

CELLULAR

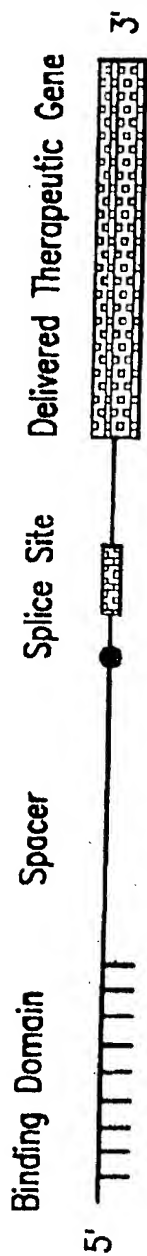
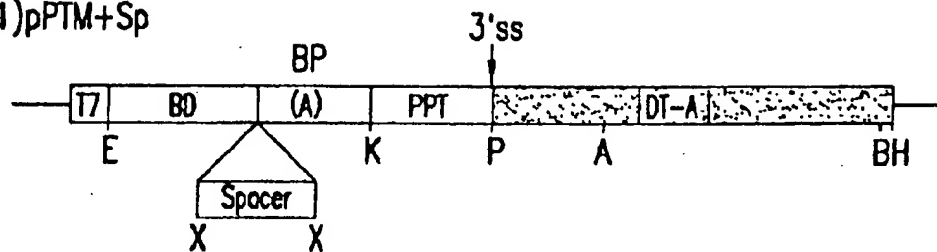


FIG.1A

(1) pPTM+Sp



(2) pPTM+Sp

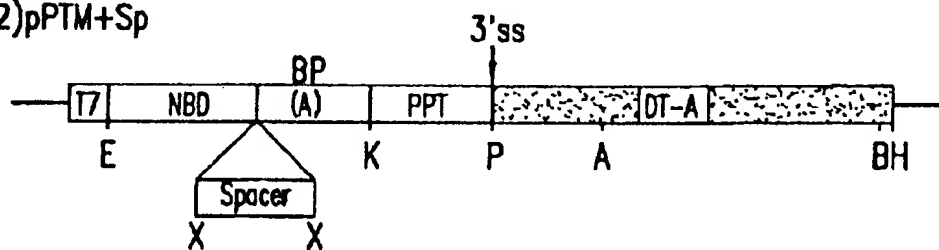


FIG.1B

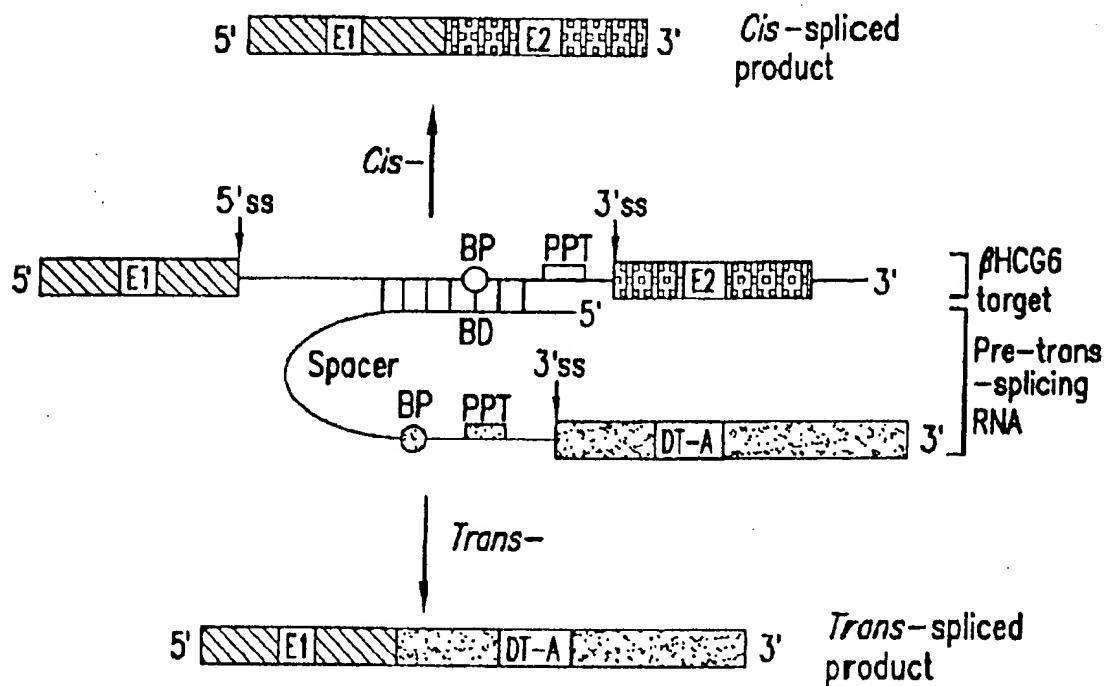


FIG.1C

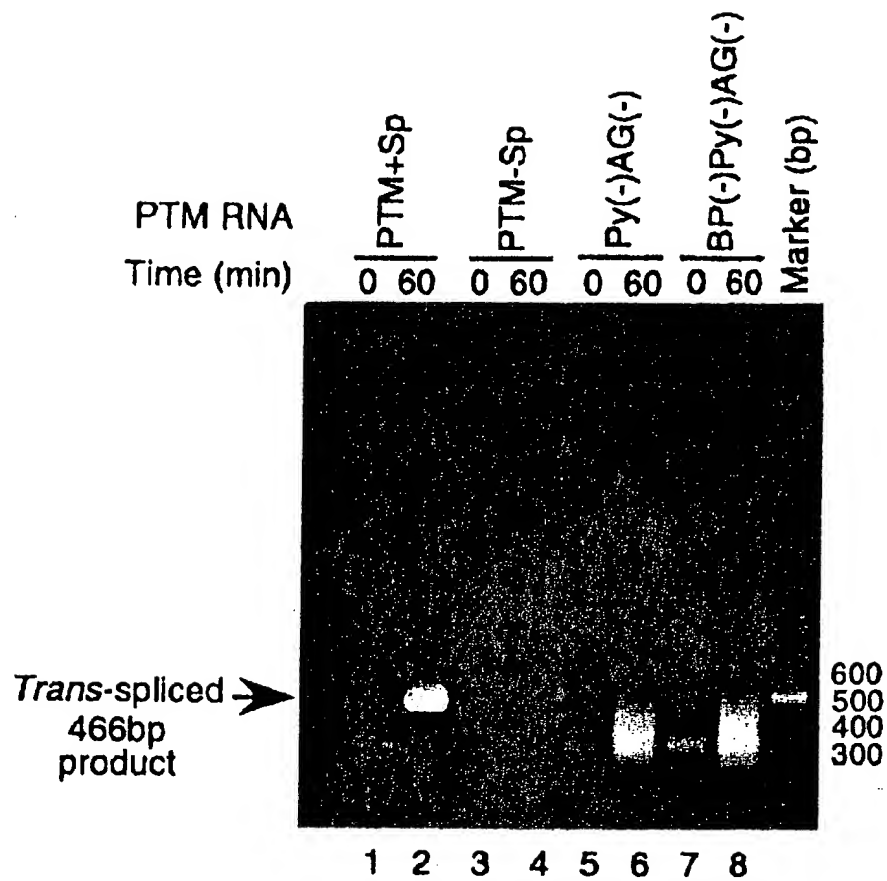
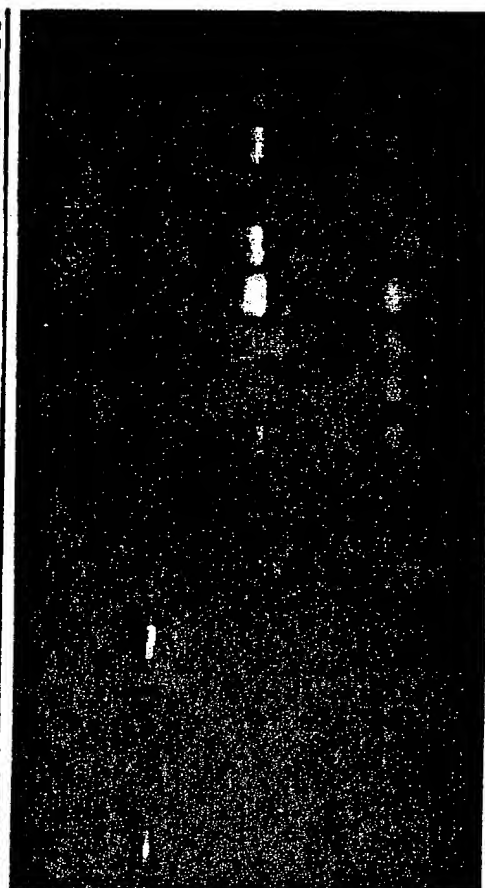


FIG.2A

Short PTM-		Short PTM+						PTM+Sp						PTM-Sp						PTM+						PTM RNA											
Marker (bp)																																					
90	60	30	90	60	30	90	60	30	90	60	30	90	60	30	90	60	30	90	60	30	90	60	30	90	60	30	90	60	30	90	60	30	90	60	30	Time (min)	



500
400
300
200
100

Trans spliced
143 bp product

Trans spliced
196 bp product

17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

FIG. 2B

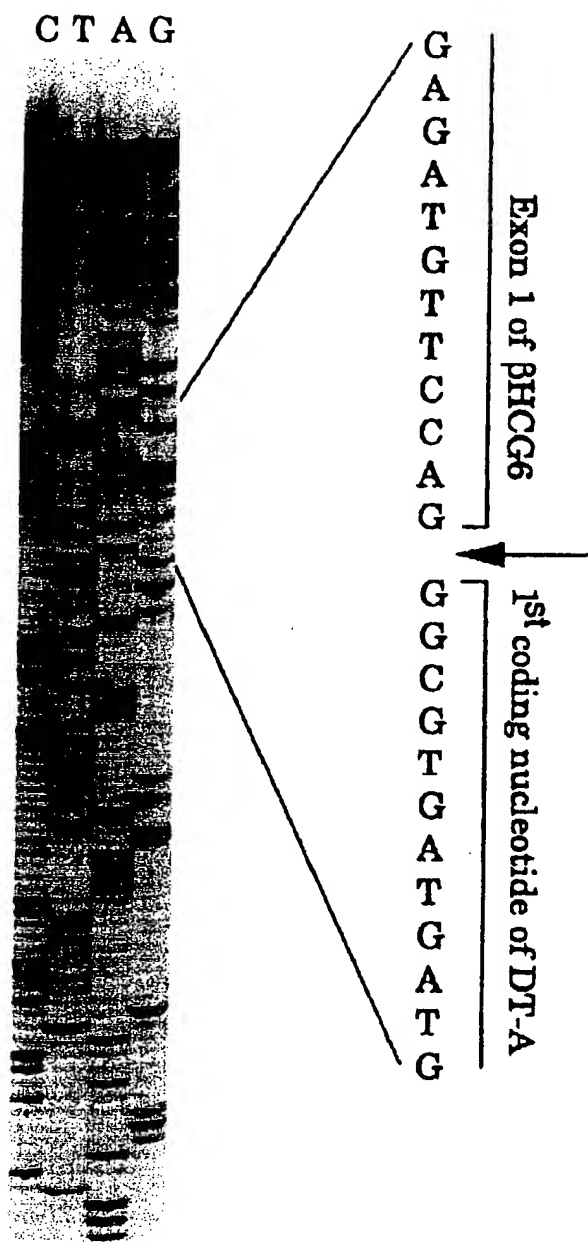
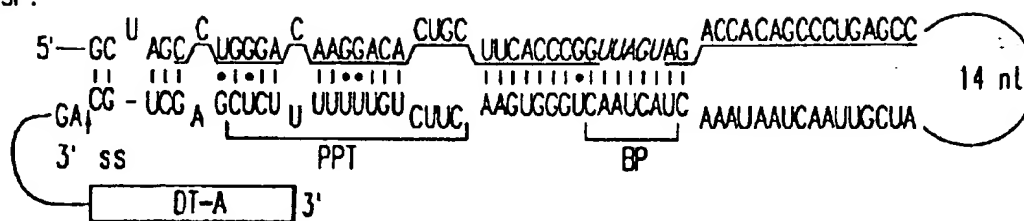


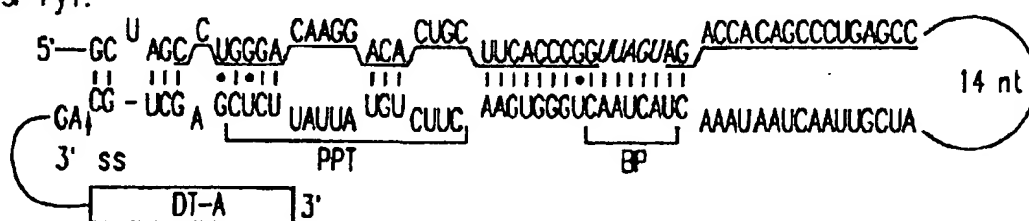
FIG.3

00000000-010000

1. PTM+SF:



2. PTM+SF-Py1:



3. PTM+SF-Py2:

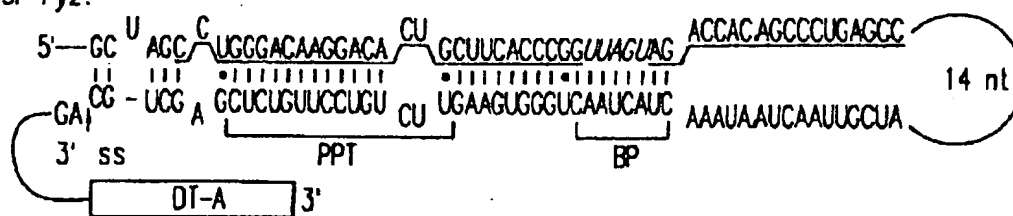


FIG.4A

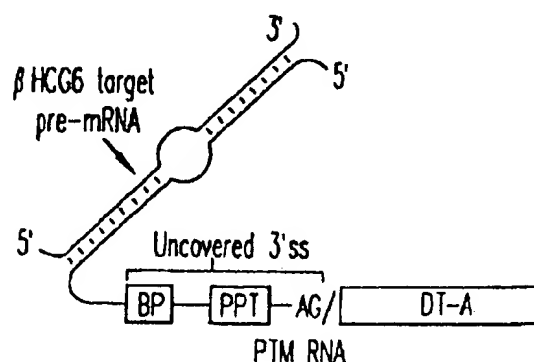


FIG.4B

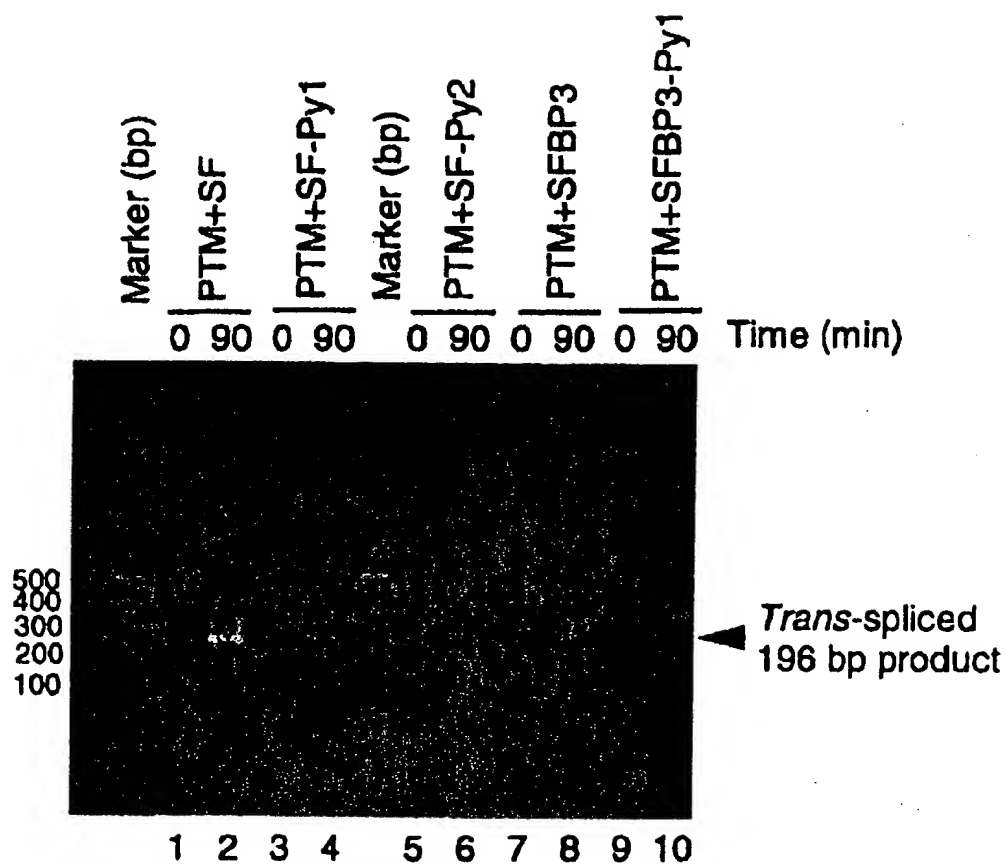


FIG. 4C

Linear PTM		Safety PTM	
Marker (bp)	<div> <div>HCGR2</div> <div>DT-3R</div> <div>β-globin-R</div> <div>β-globin-F</div> <div>HCGR2</div> </div>	<div> <div>HCGR2</div> <div>DT-3R</div> <div>β-globin-R</div> <div>β-globin-F</div> <div>HCGR2</div> </div>	<div>Forward Primer</div>
			<div>Reverse Primer</div>

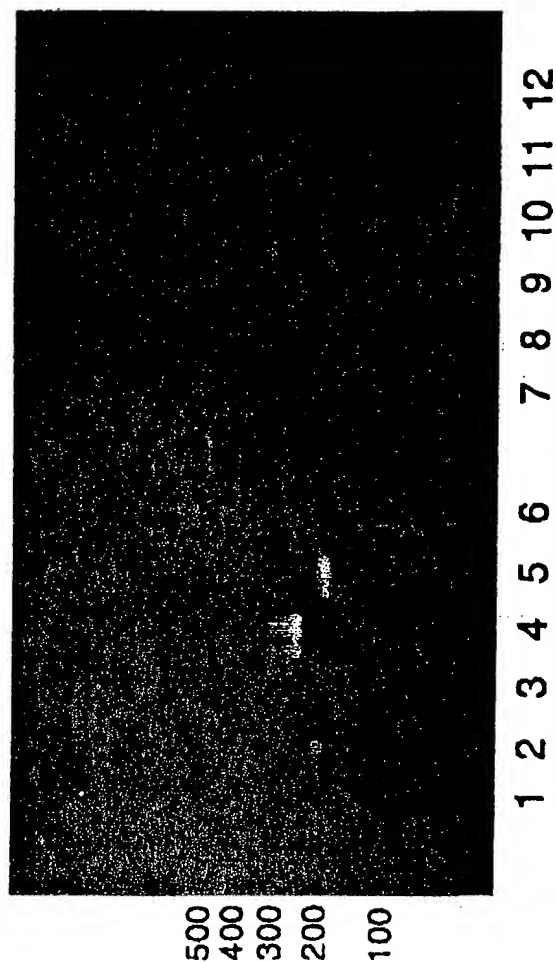


FIG. 5

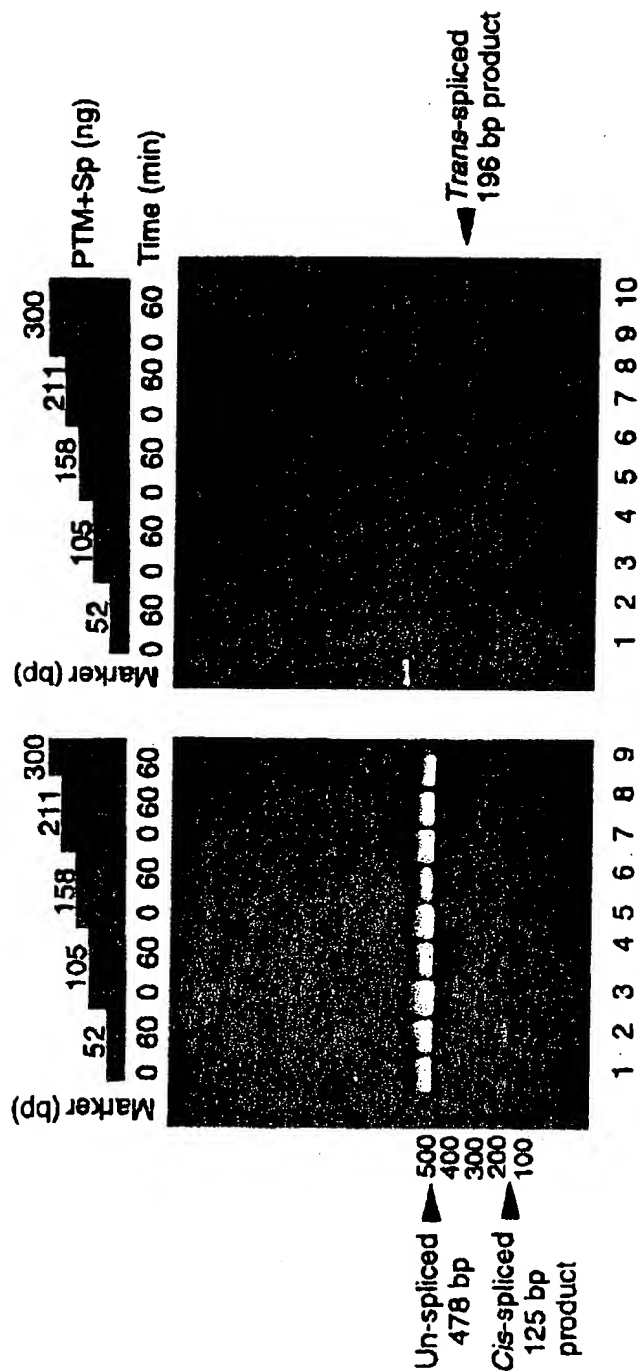


FIG. 6A

FIG. 6B

20070707 03002000

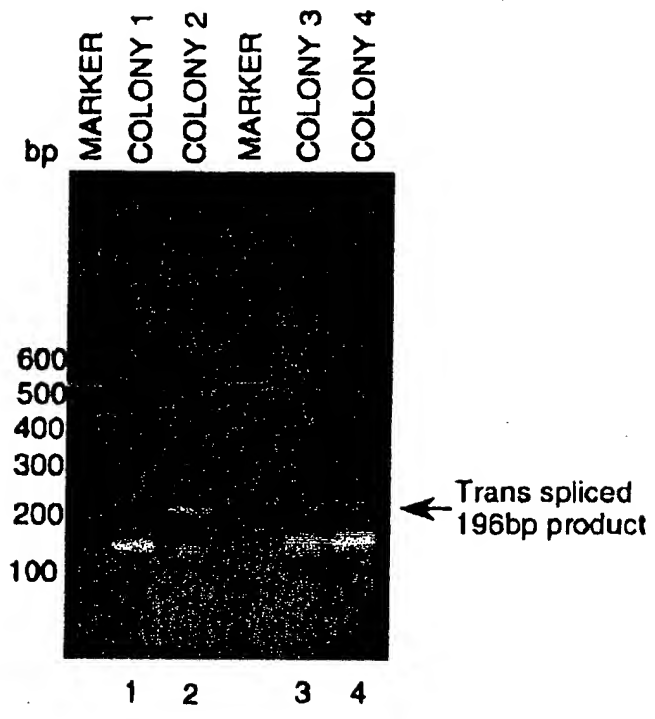


FIG.7A

EXON 1 OF β HCG6 ↑
5'-CAGCGGACGCACCAAGGATGGAGATGTTCCAG-GGGCTGATGATGTTGTT
↑ 1ST CODING NUCLEOTIDE OF DT-A
GATTCTTCTTAATCTTTTGTGATGGAAACTTTTCTTCGTACCAACGGGACTA
AACCTGGTTATGTAGATTCCATTCAAAA-3'

FIG. 7B

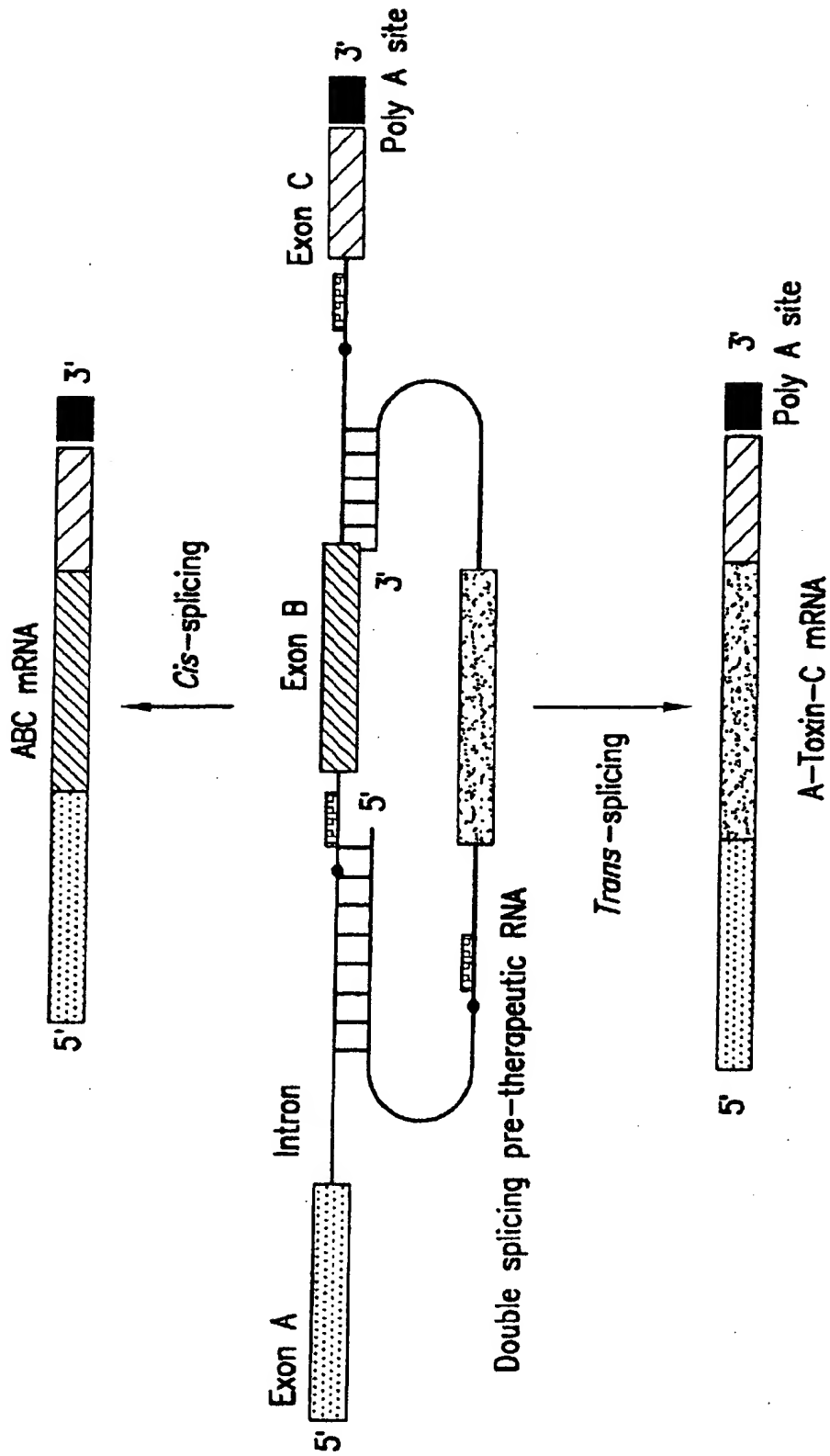
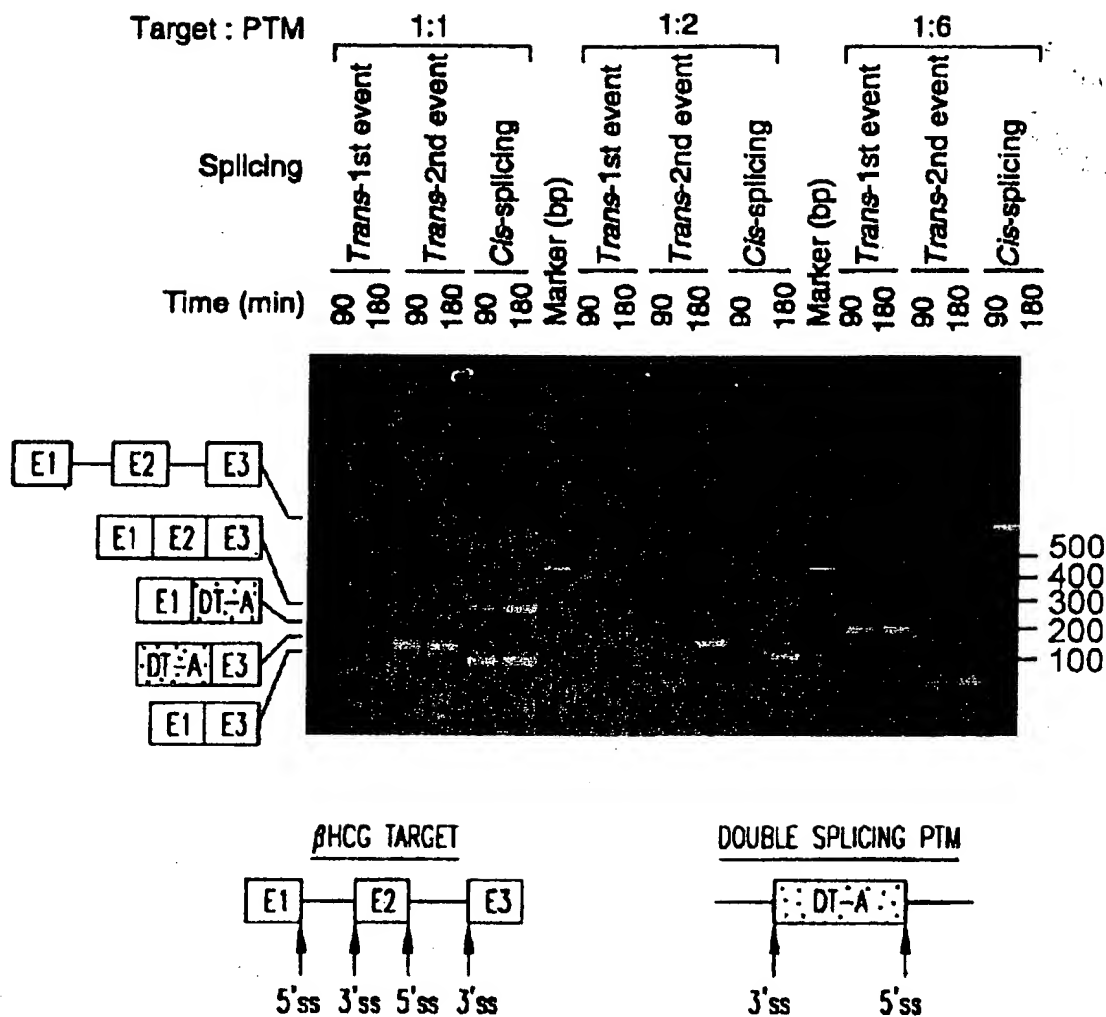


FIG.8A



Cis-spliced products

E1 E2 E3 = NORMAL *cis*-SPLICING (277bp)

E1 E3 = Exon SKIPPING (110bp)

Trans-spliced products

E1 DT-A = 1st EVENT, 196bp. *Trans*-SPLICING BETWEEN 5' ss OF TARGET & 3' ss OF PTM.

DT-A E3 = 2nd EVENT, 161bp. *Trans*-SPLICING BETWEEN 3' ss OF TARGET & 5' ss OF PTM.

FIG.8B

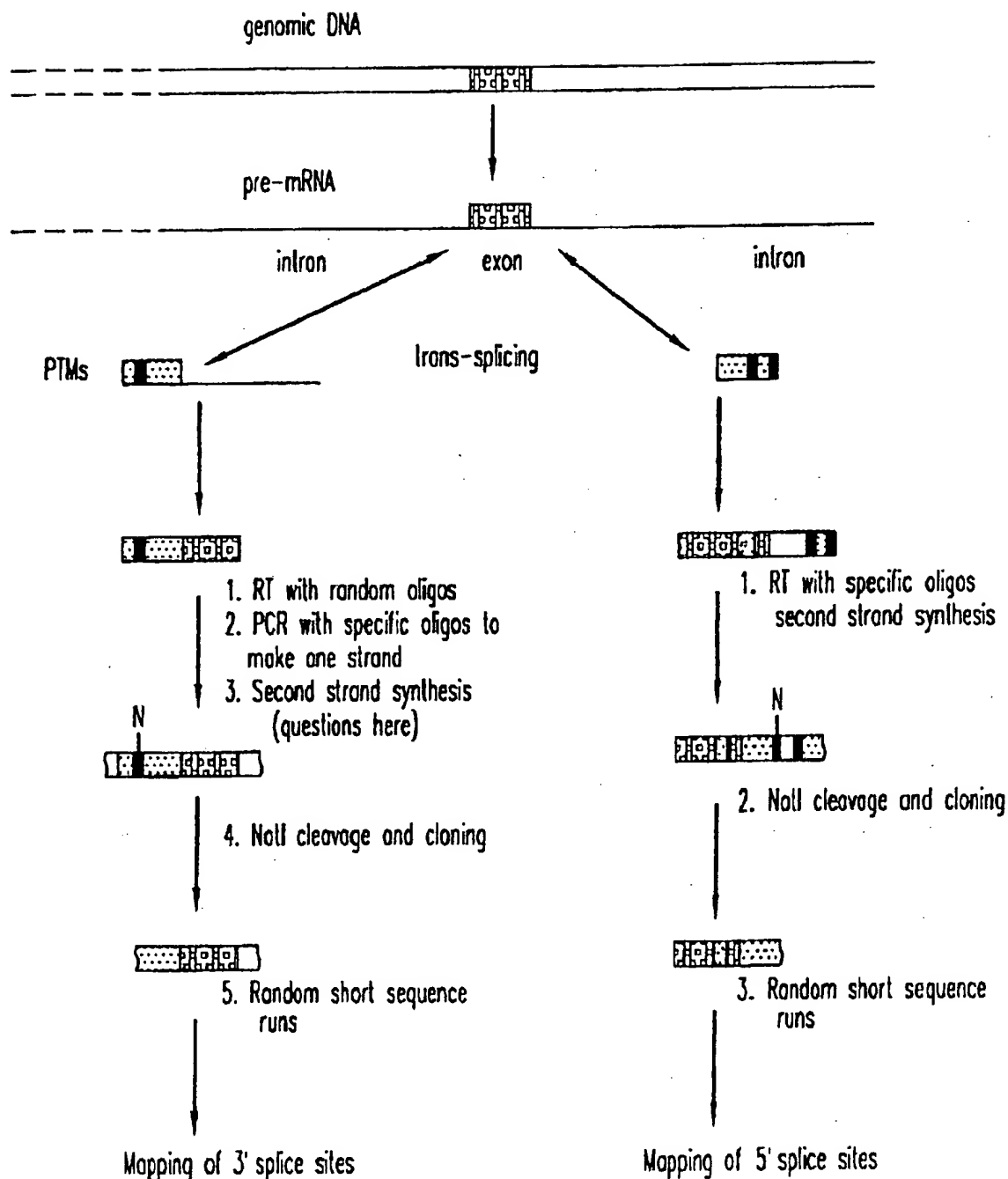


FIG.9

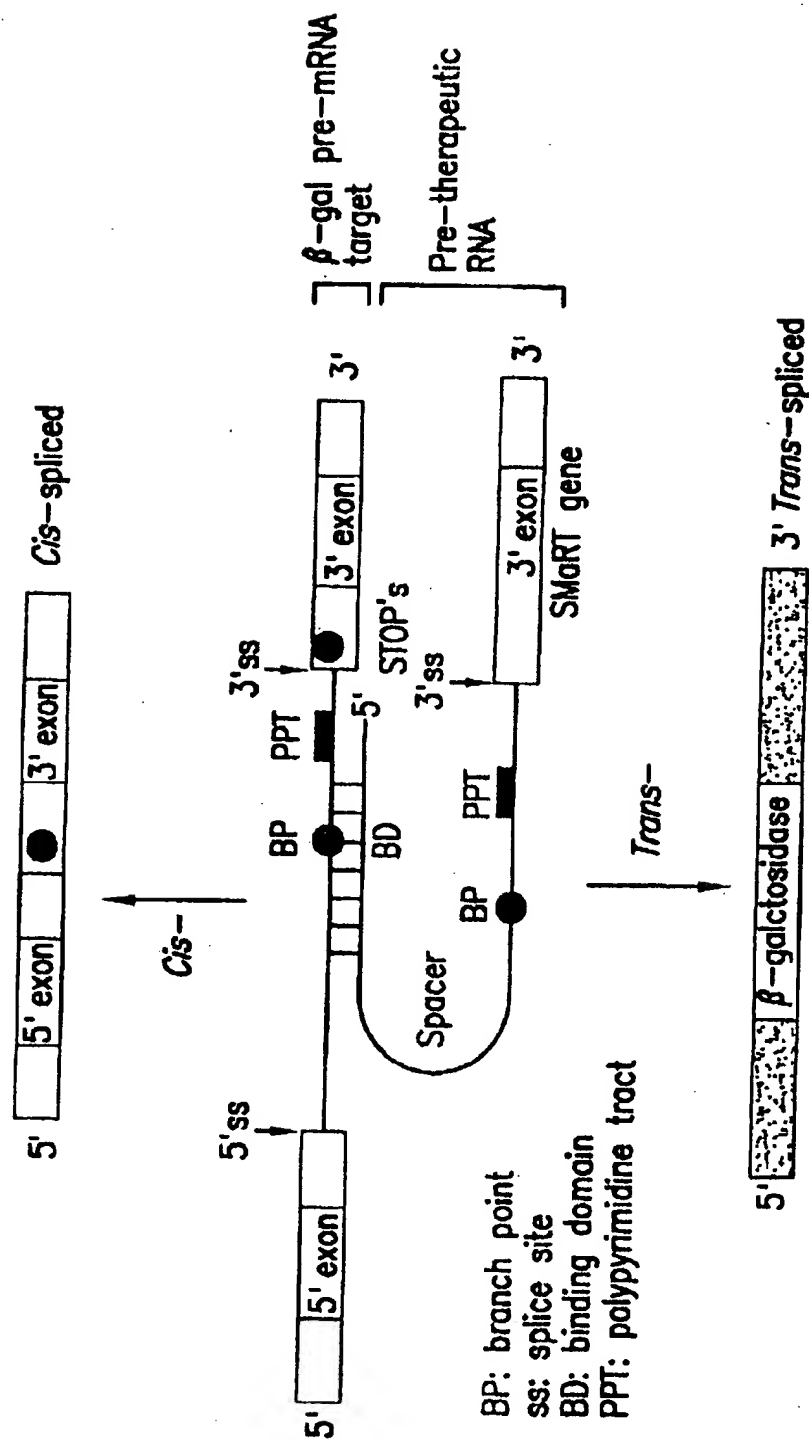


FIG. 10B

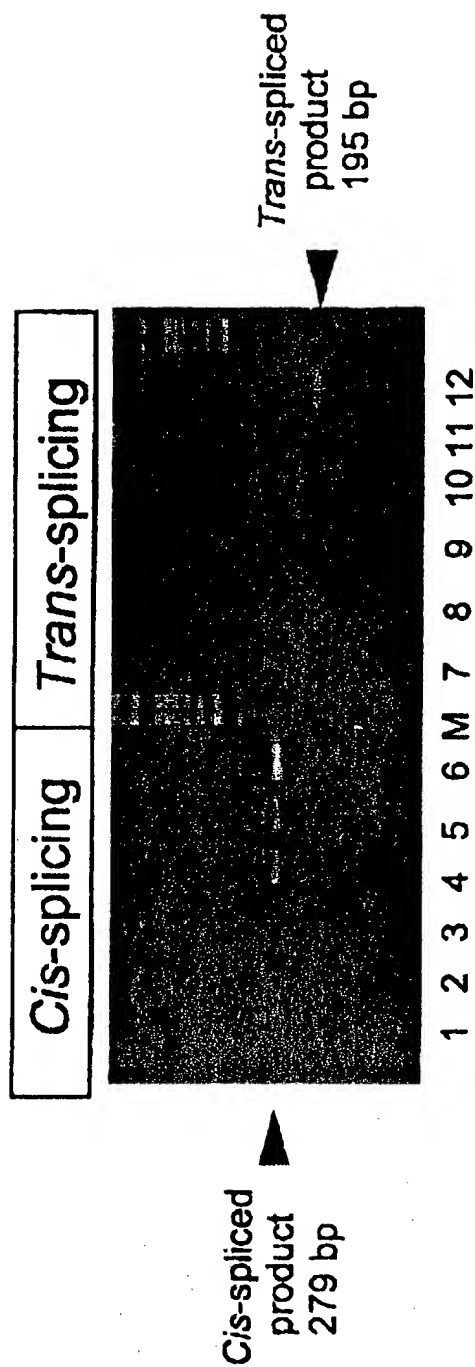


FIG.11A

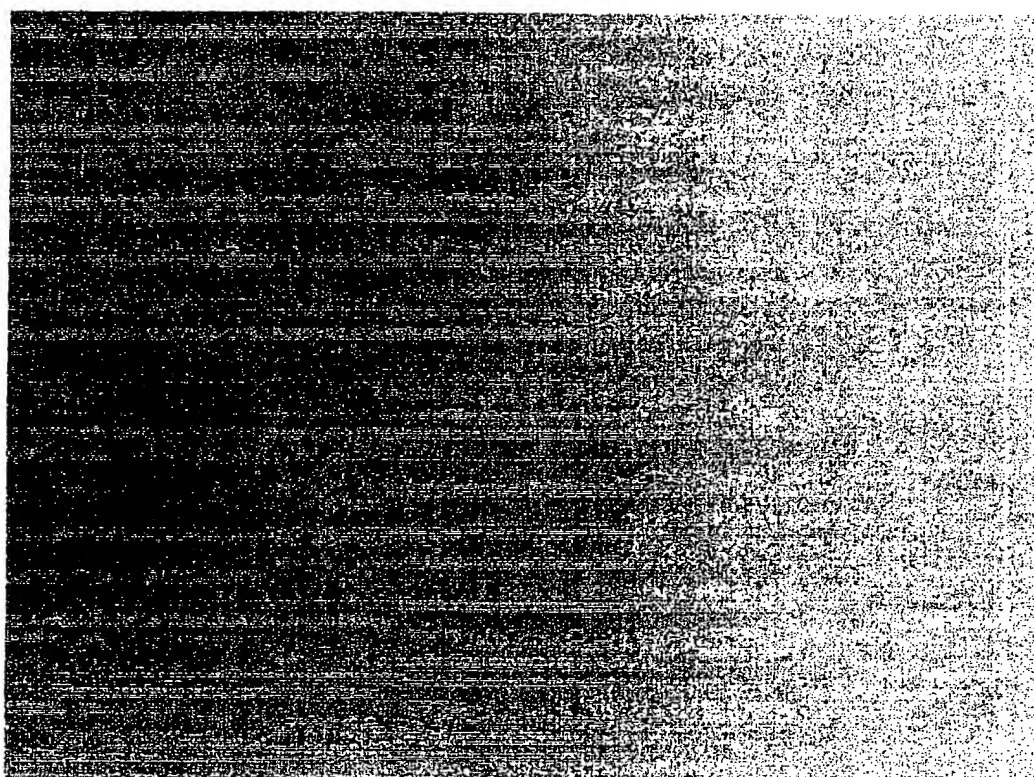
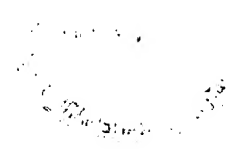


FIG. 11B

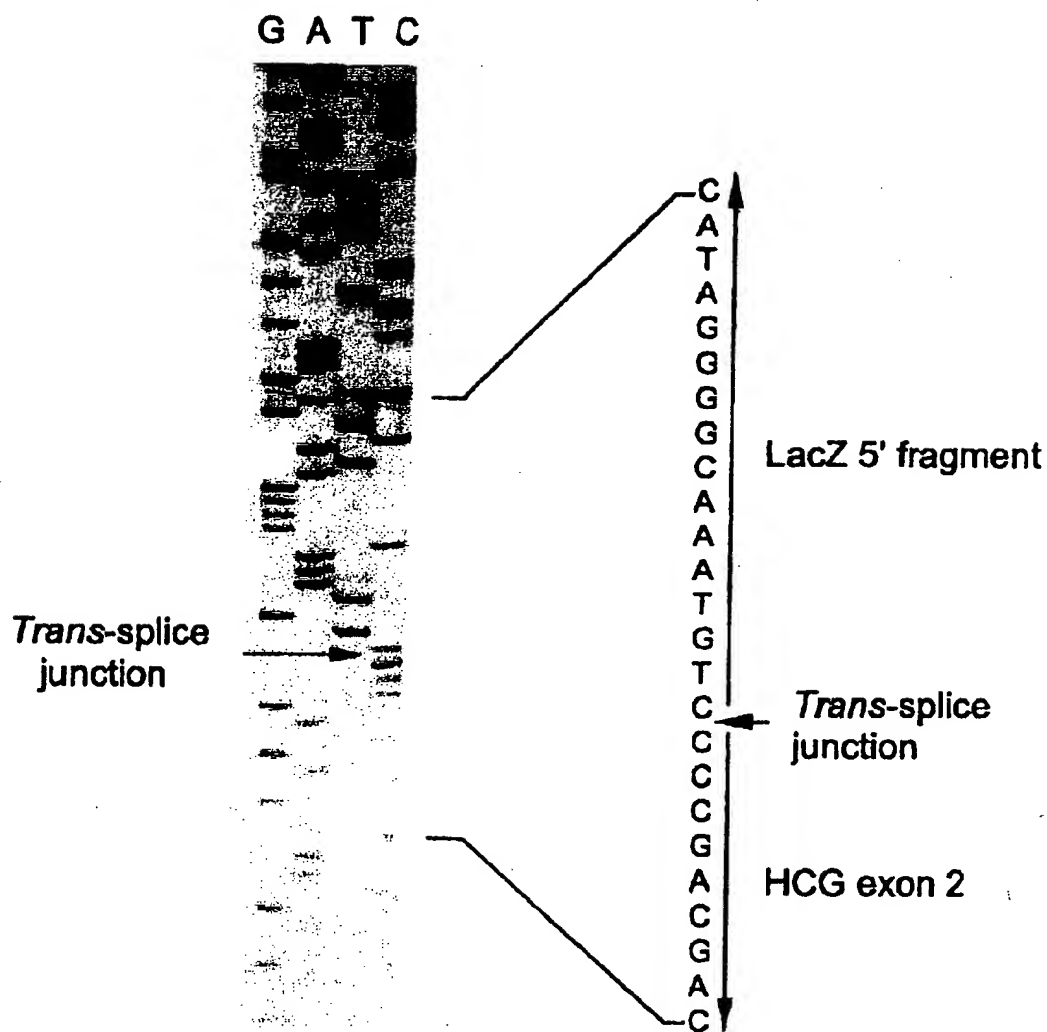


FIG.12A

Biolac-TR1

GGCTTTGGCTACCTTCGAGAGACGGCCCGCTGATCCTTTGGGAATACGGCCACGGGATGGGTAAACAGTCTTG

Splice junction

GGGGTTTCGGCTAAATAC TGGCAGCGCTTCGTCAGTATCCCGTTTACAG/GGCGGCTTCGTCCTAATAATG

GGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAACGGCAACCGTGGTCGGCTTACGGCGGTGATTT

TGGCGATACGCGAAGCATGCCAGTTCGCTGTA TGAACGGCTCGTCTTTGCCGACGGCAGCGGCATCCAG
Lac--TR2

2. NUCLEOTIDE SEQUENCES OF THE *trans*-SPLICED PRODUCT (195 bp)

Bialac-TRI

GGCTTTCGCTACCTGGAGAGACGCCCGCGCTGATCCTTTGGCAATACGCCCAAGCGATGGGTAAACAGTC TTGG

Splice junction

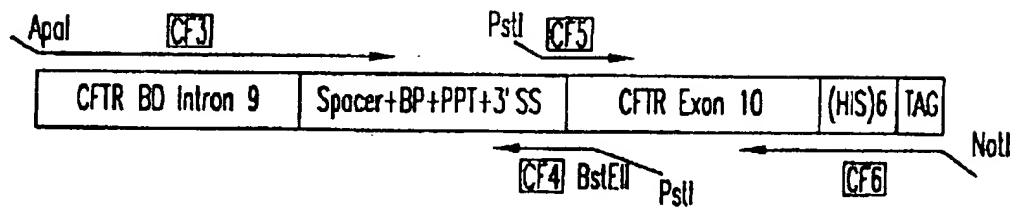
CGGTTTCGCTAAATACTGGCAGCGTTTCGTCAGTATCCCGTTTACAG/CGGCTGCTGCTGTTGCTGCTGCT

HCGR2

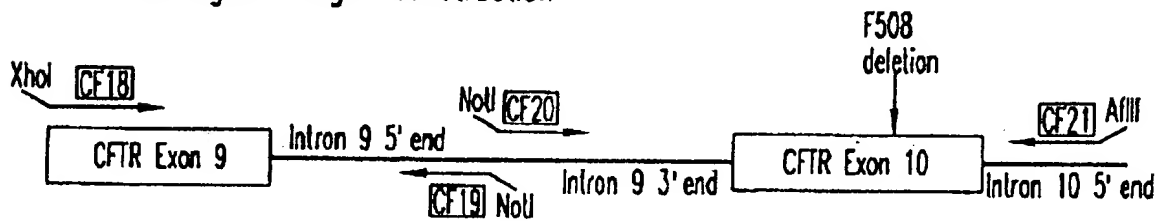
GAGCATGGGCGGACATGGGCATCCAGGAGCCACTTCGGCCACGGTGC GG
TCGGT

FIG. 12B

CFTR Pre-therapeutic molecule (PTM or "bullet")



CFTR mini-gene target-construction



Trans-splicing Repair

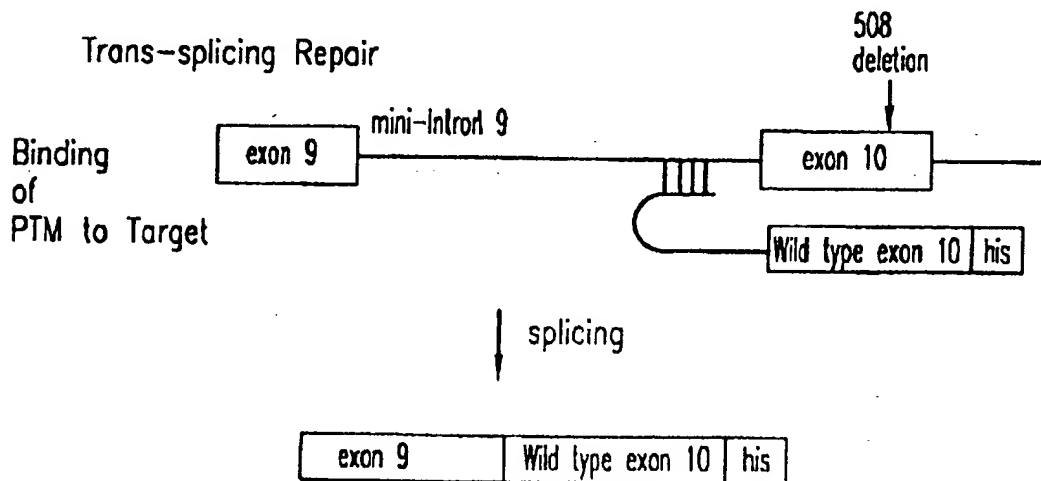


FIG.13

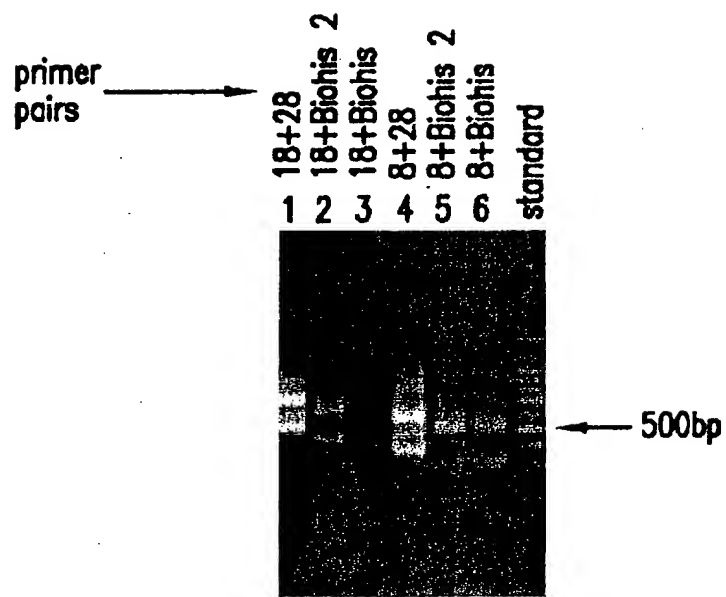


FIG.14

DNA sequence 500 b.p. GCTAGCGTTTAA ... TGCCACTCCAC linear

Positions of Restriction Endonucleases sites (unique sites underlined)

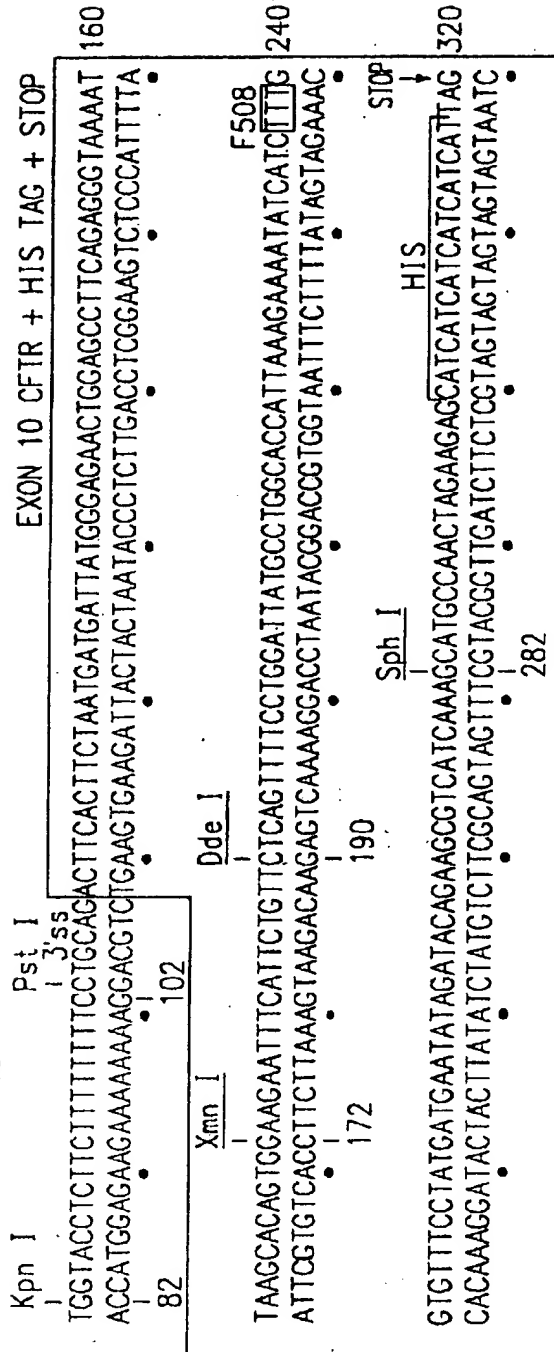
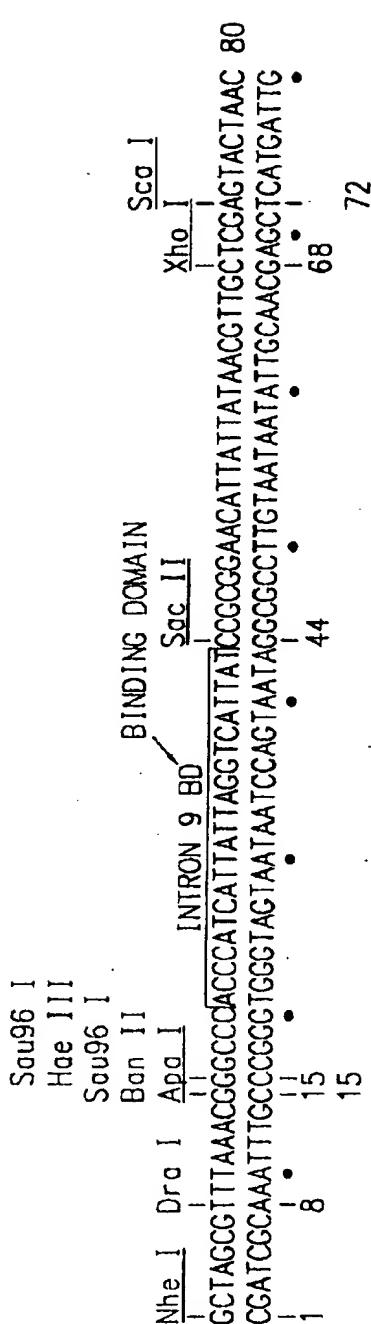
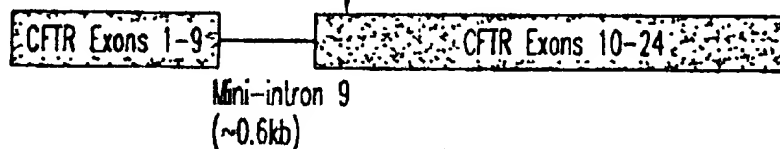


FIG.15A



+

CFTR Target
(mini-gene)



Cotransfect PTM and target molecules in HEK 293 cells
and detect repaired CFTR mRNA by RT-PCR.

Repaired
CFTR mRNA

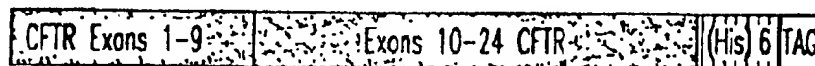


FIG.16

CFTR BD intron 9	Spacer+BP+PPT+3'SS	CFTR exon 10	Spacer+BP+PPT+5'SS	CFTR BD intron 10
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Double Splicing
PTM

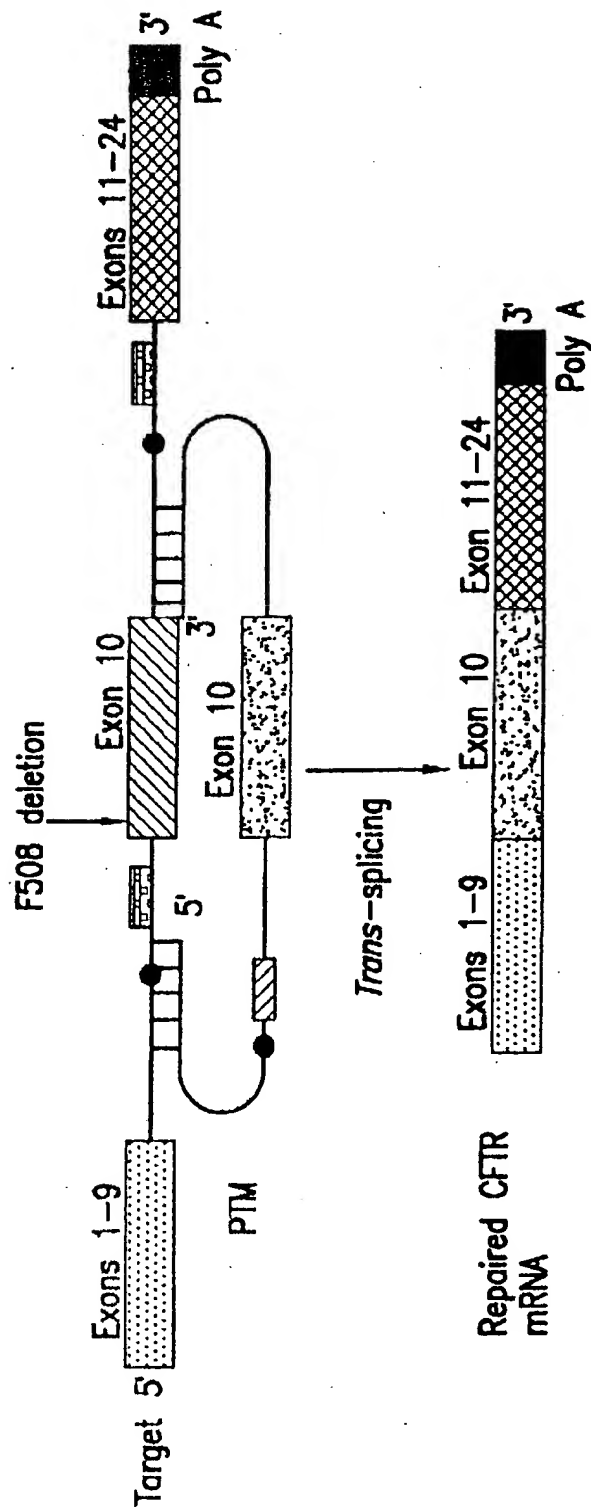


FIG.17

DOUBLE TRANS-SPICING PTMs

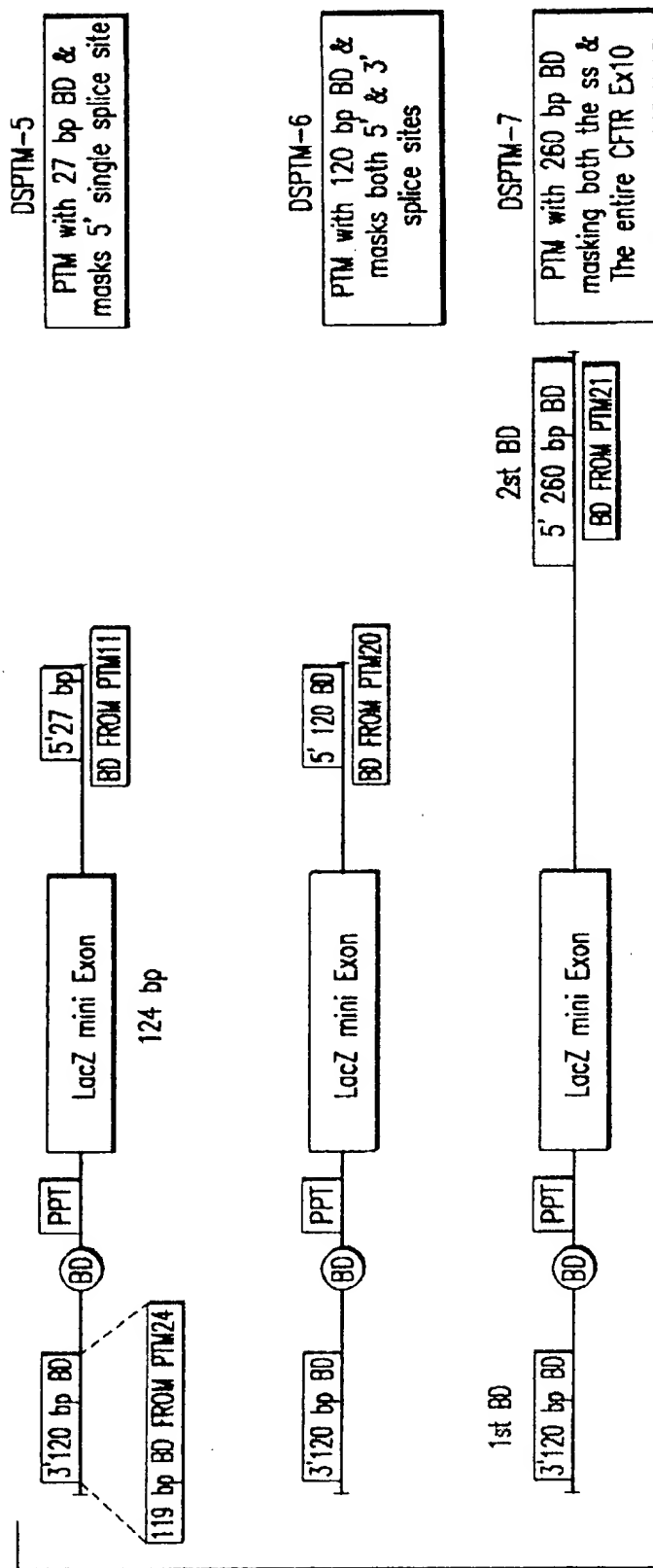
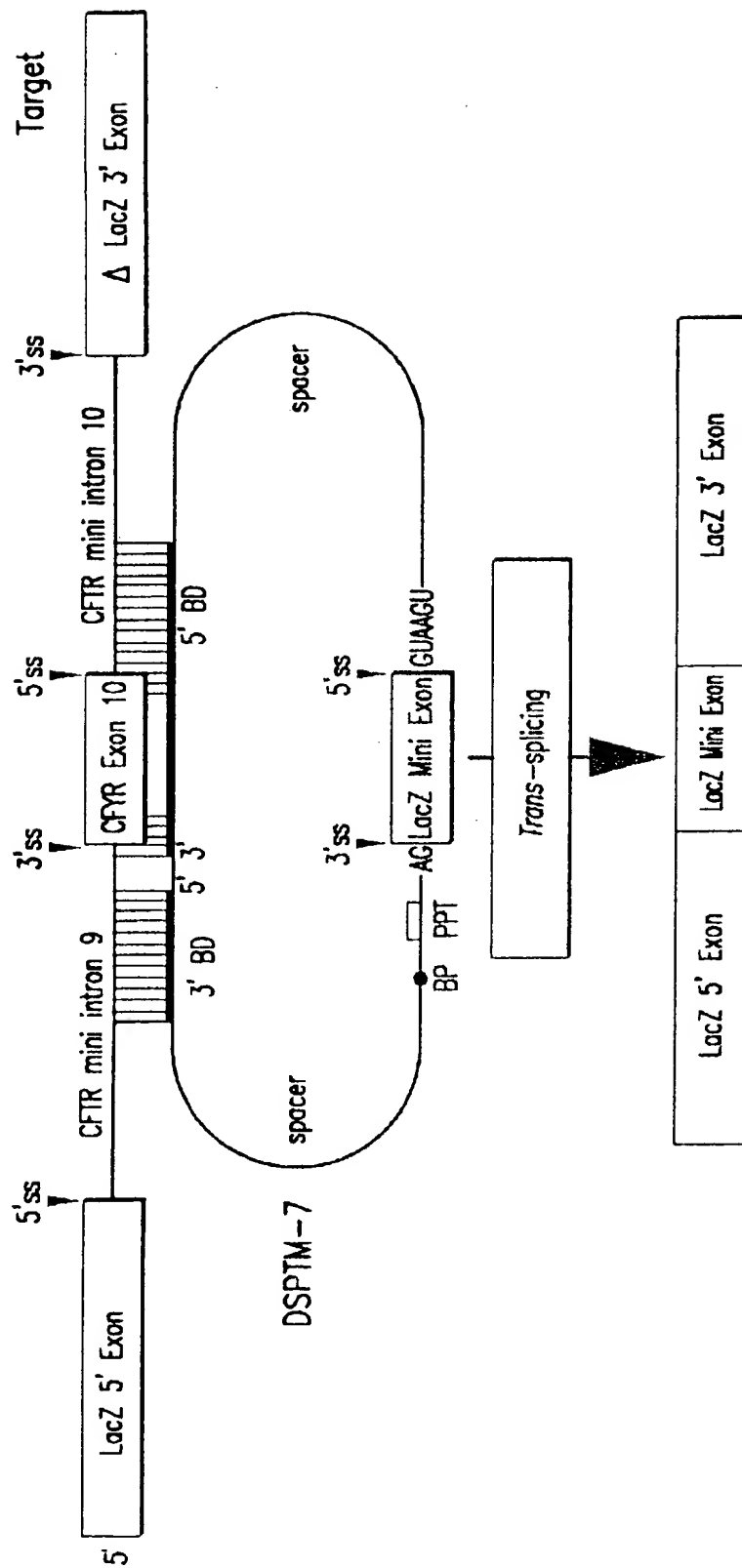


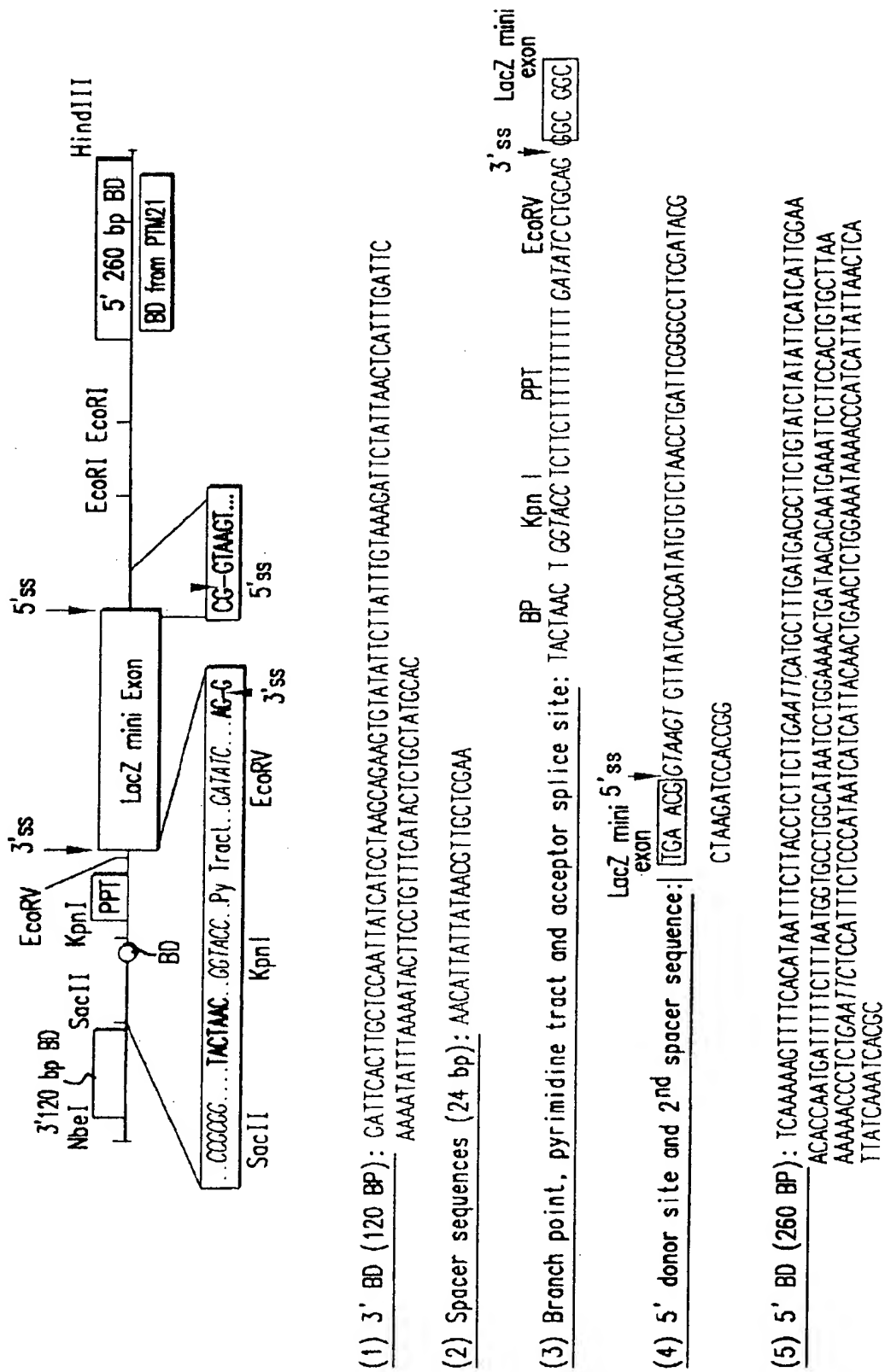
FIG.19

DOUBLE TRANS-SPLICING β -GAL MODEL

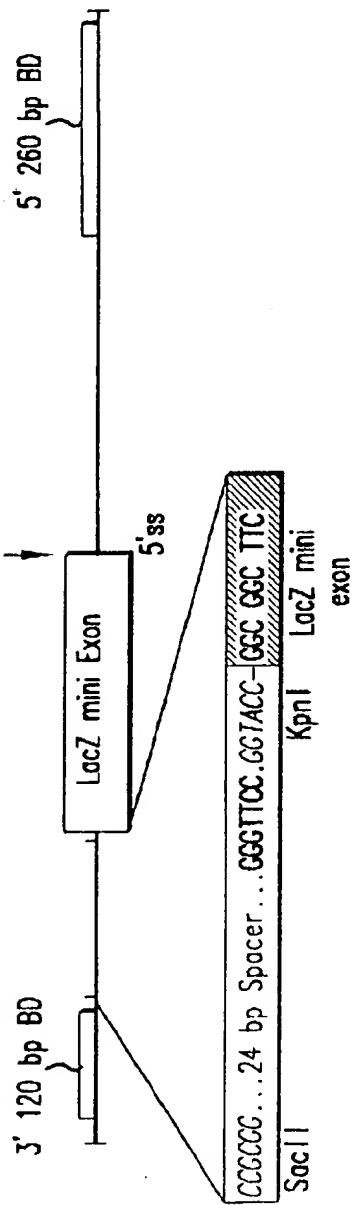


Repaired LacZ mRNA

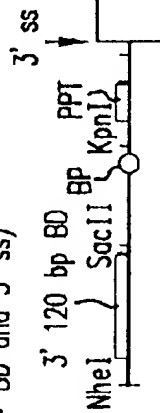
FIG.20



DSPTM8: (Δ 3' ss: 3' splice elements i.e. BP, PPT & AG dinucleotide has been deleted and replaced with random sequences, but still has the functional 5' splice site)



PTM29 (lacks 2nd BD and 5' ss)



PTM30 (lacks 1st BD and 3' ss)

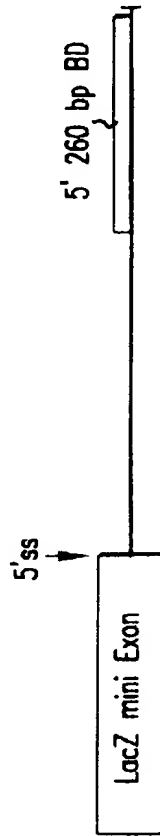


FIG.22

Mutants

2020-05-06

ACCURACY OF DOUBLE TRANS-SPlicing REACTION

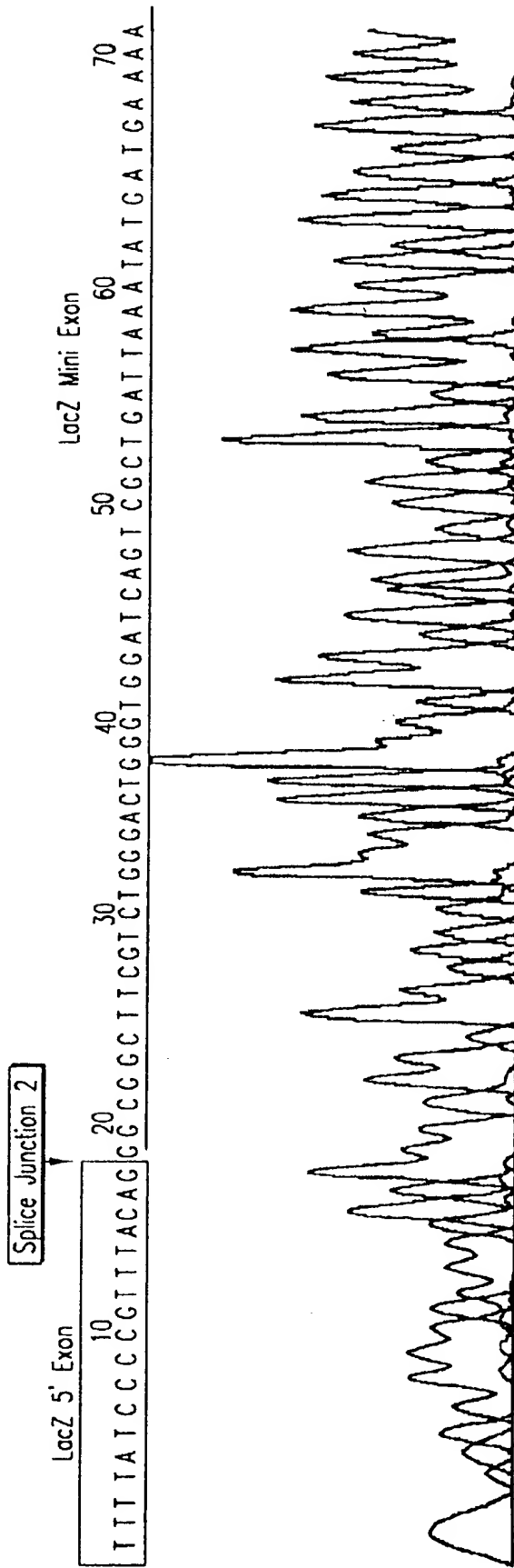


FIG.23A

ACCURACY OF DOUBLE TRANS-SPICING REACTION

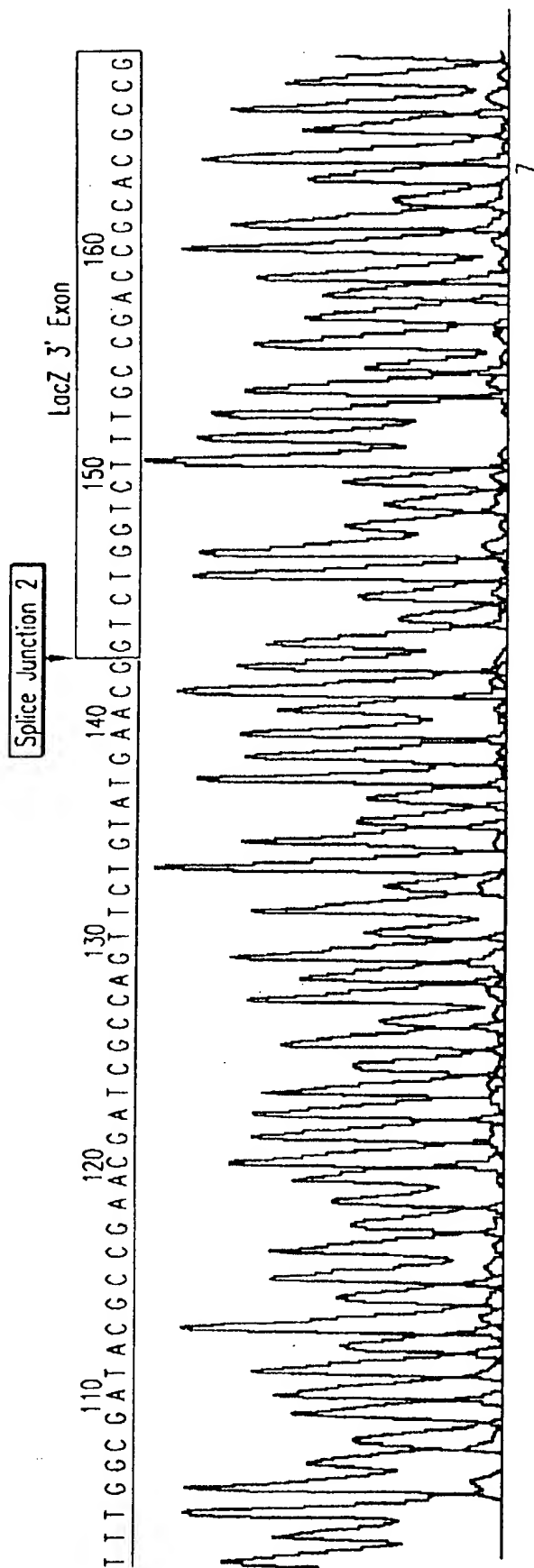


FIG.23B

Double Trans-splicing Produces Full-length Protein

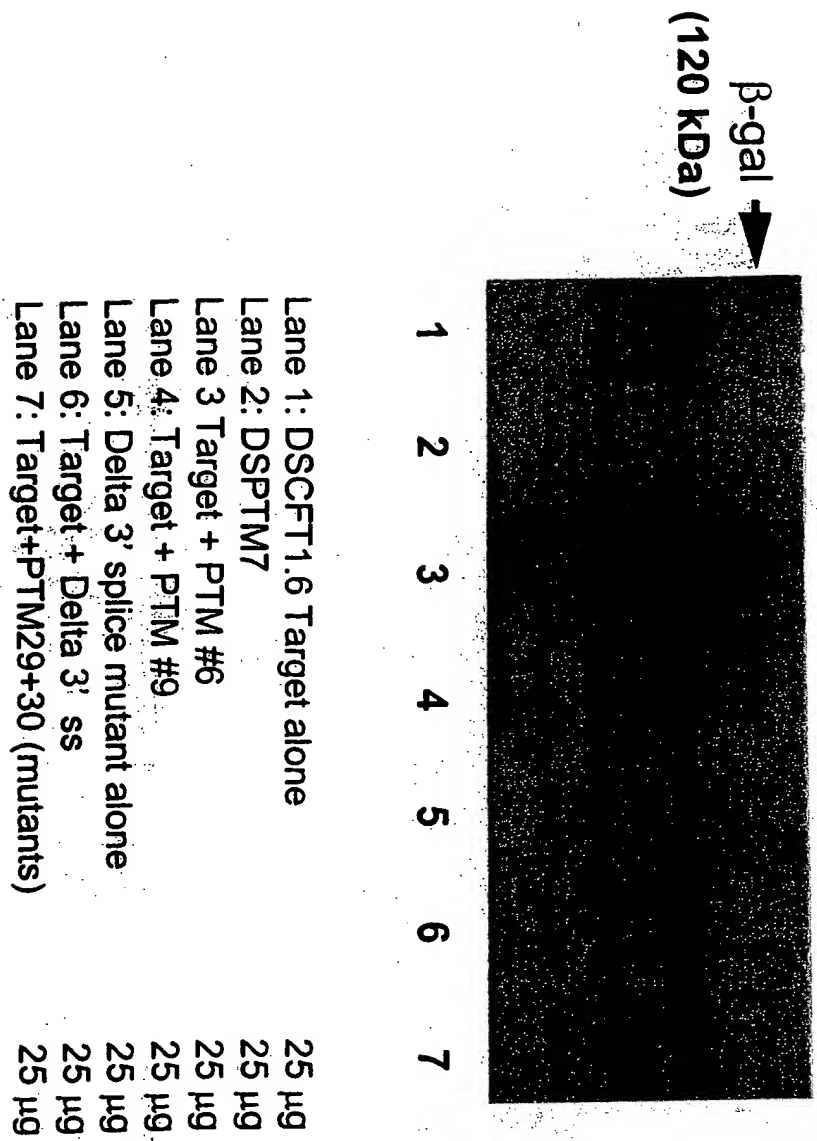


Figure 24

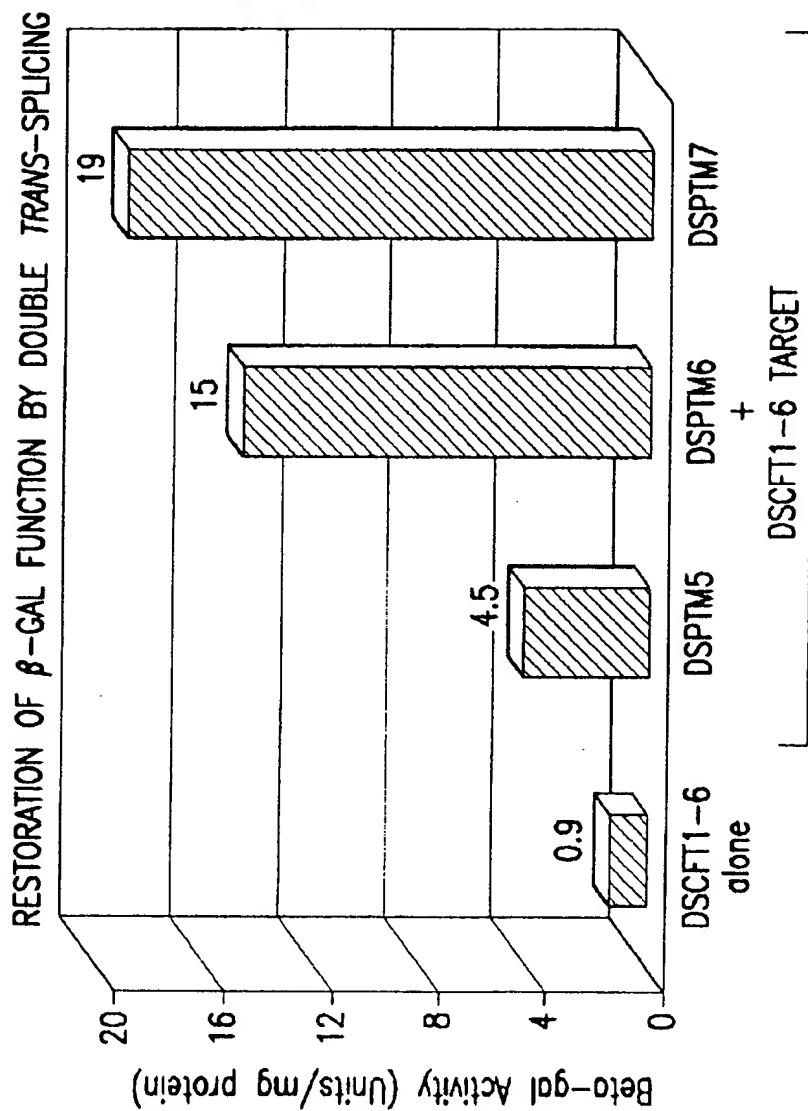
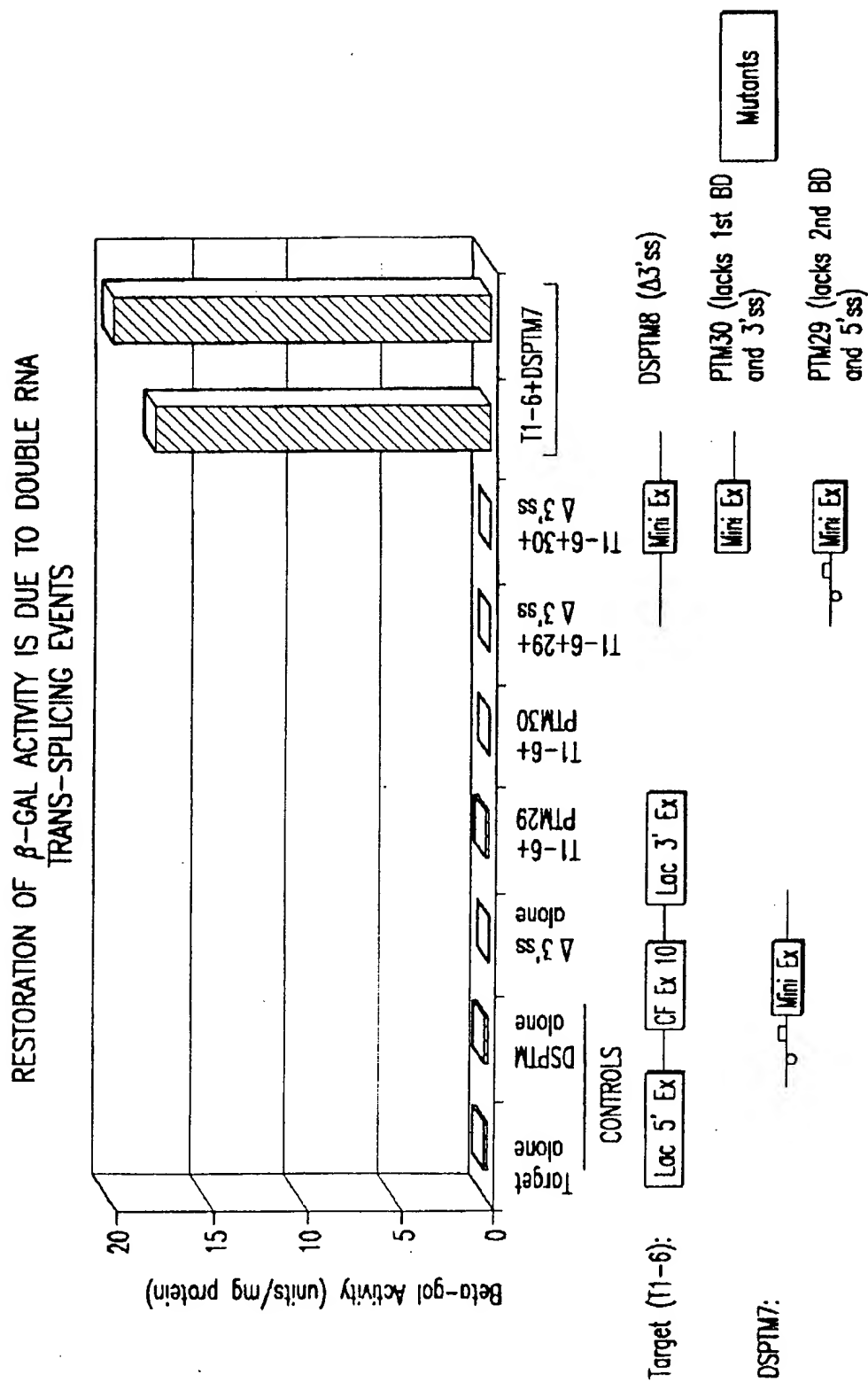
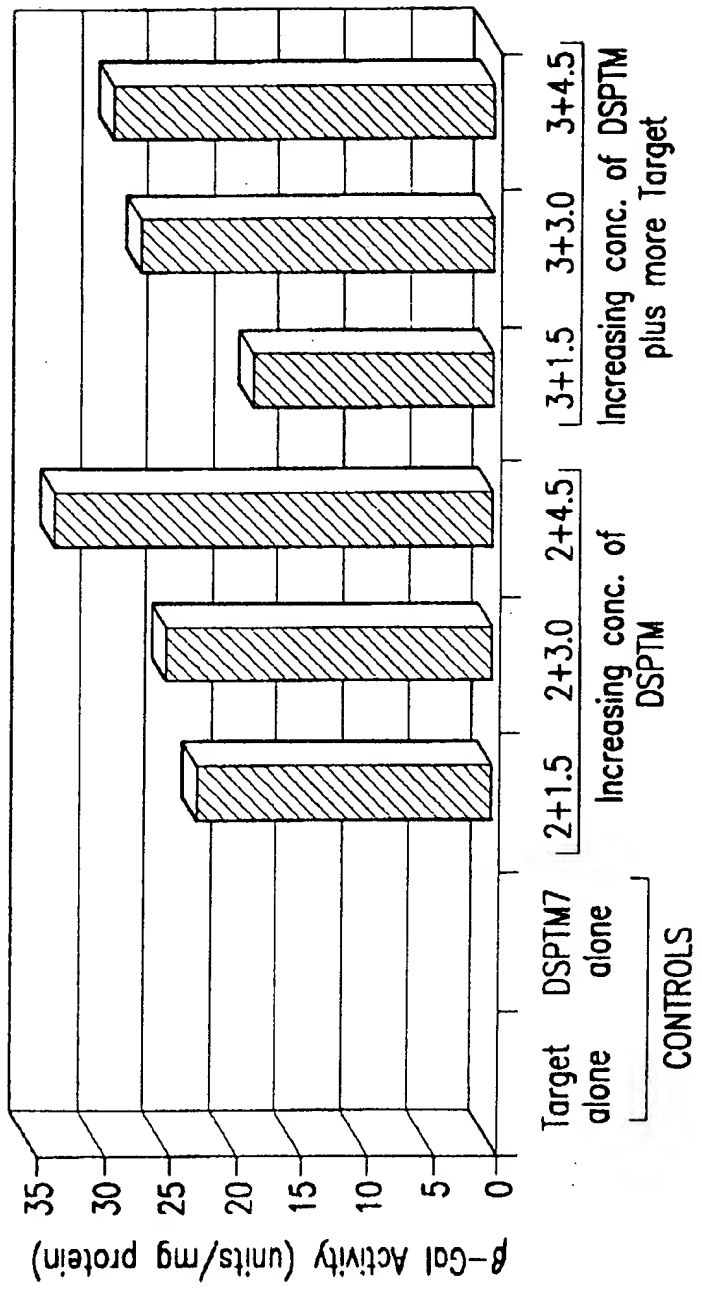


FIG.25



DOUBLE TRANS-SPLICING: TITRATION OF TARGET & PTM



The current level of beta-gal activity due to double *trans*-splicing is ~ 1-1.5% of the best single splice model (3' exon replacement)

FIG.27

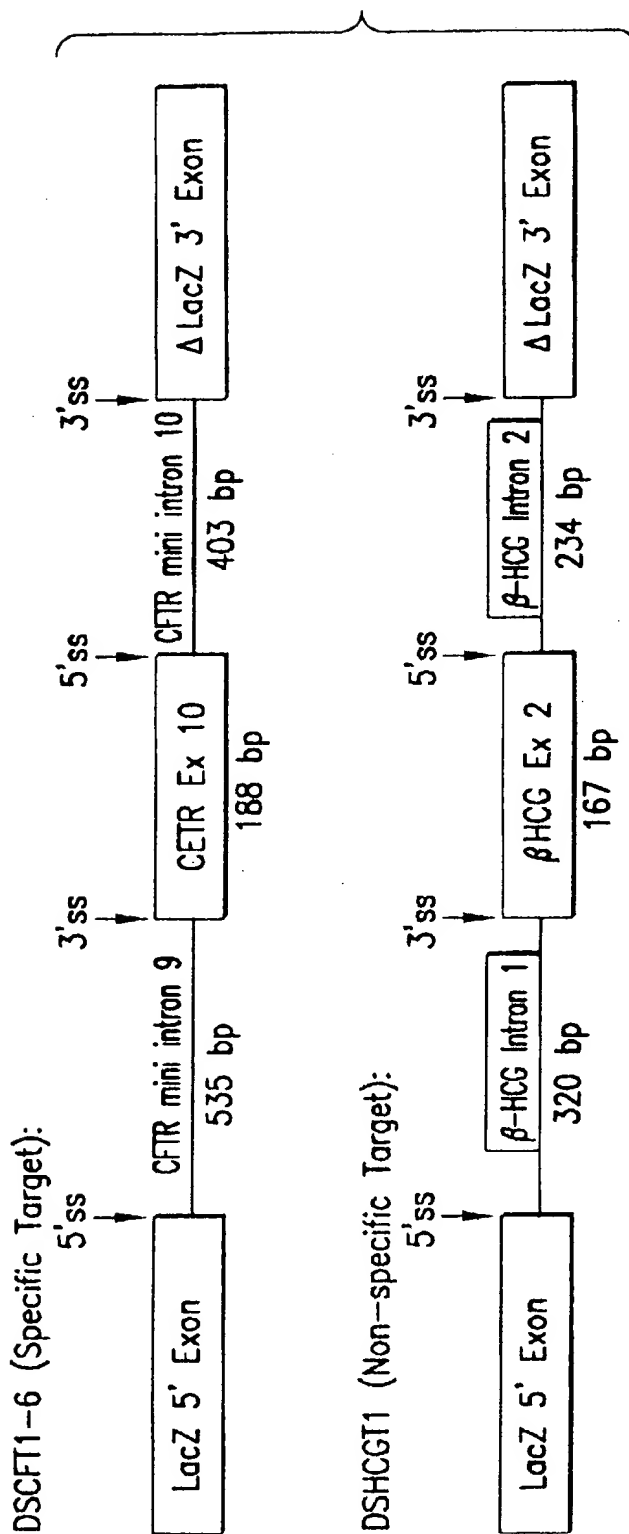
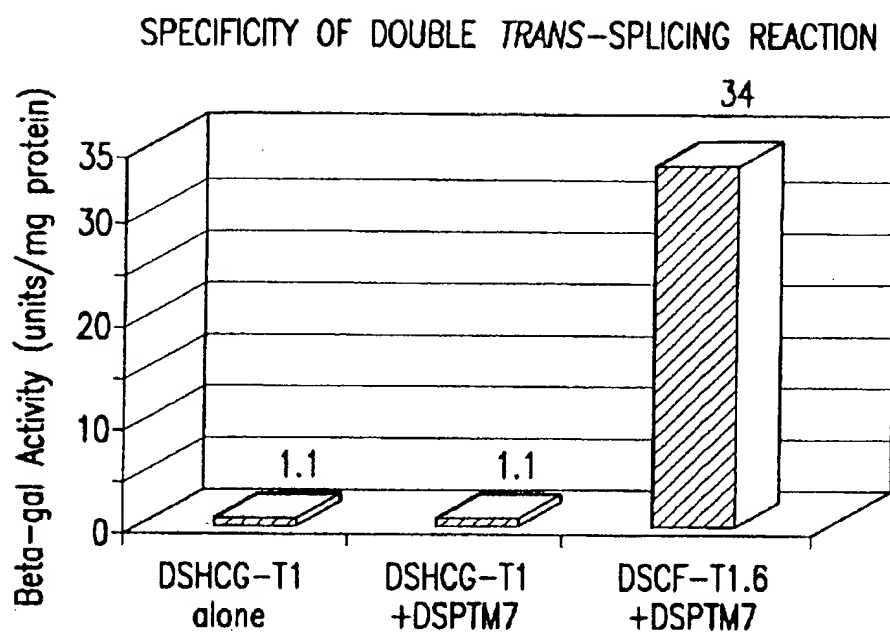


FIG.28



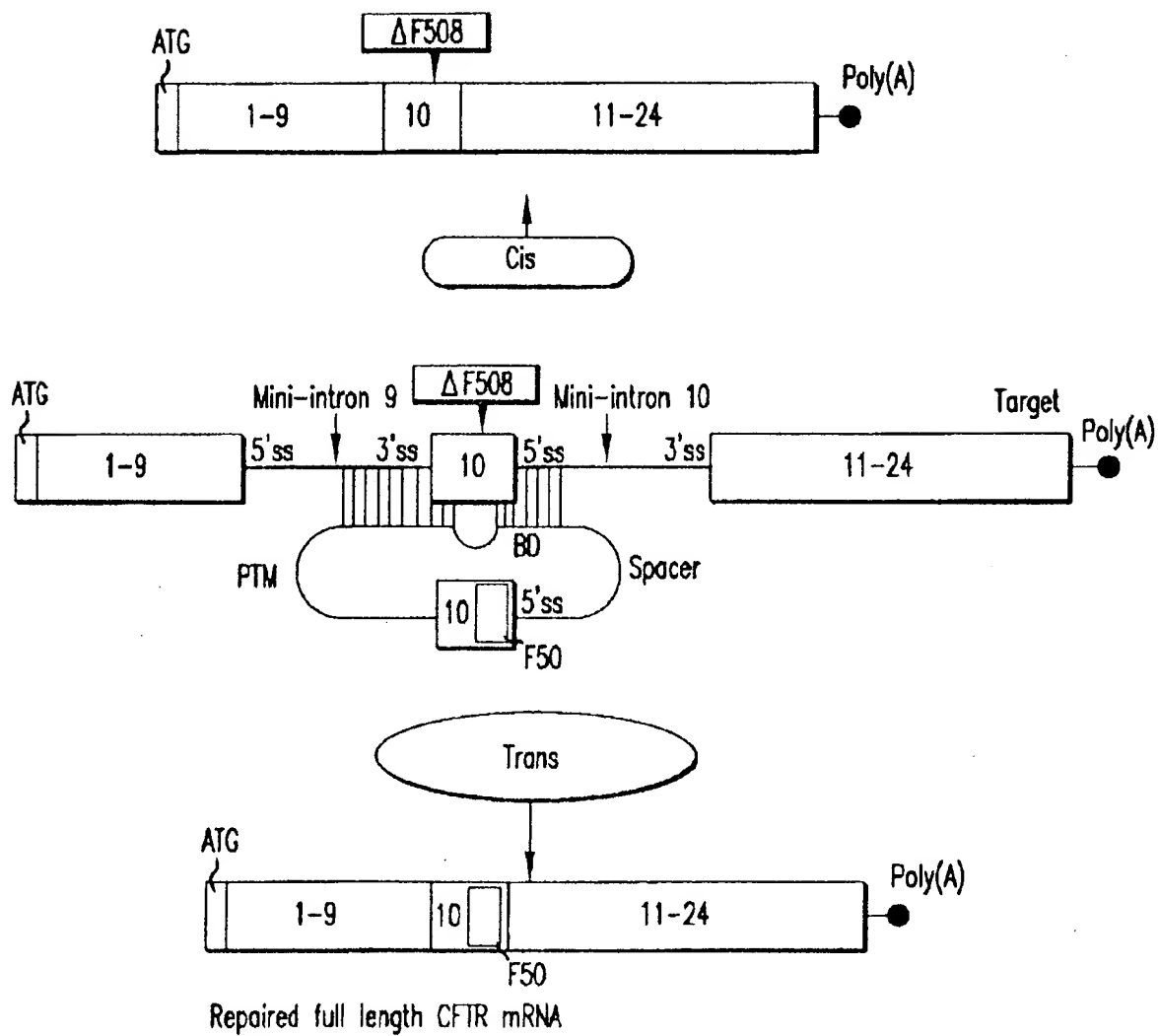
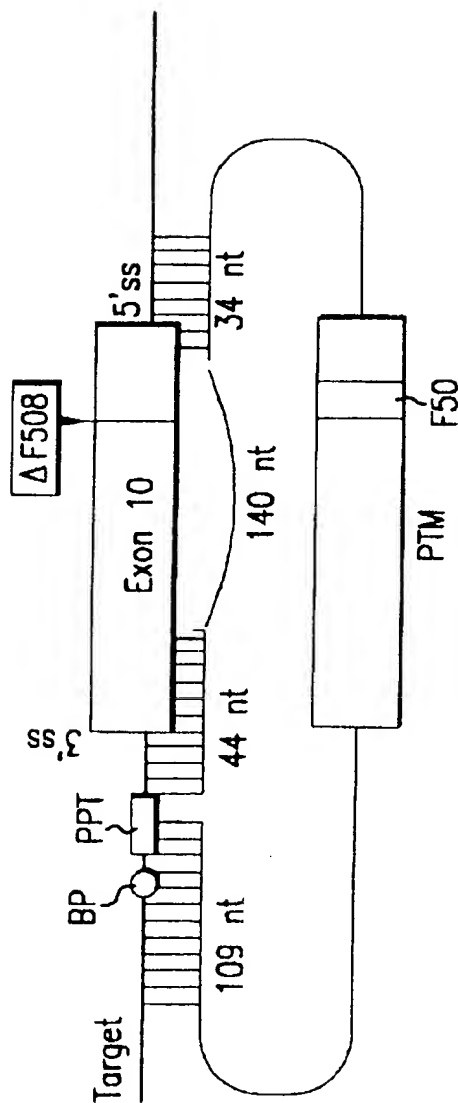


FIG.30

PTM with a long binding domain masking
two splice sites and part of exon 10
in a mini-gene target



ACGAGCTTGCATCATGATGATGCGGAGTTAGAACCAAGTGAAGGCAAGATCAACATTCGG
GCCGATCAGCTTTTCAGGCCAATTCAGTGGATCATGCCCGGTACCATCAAGGAGAAATAT
CTTCGGCGTCAGTACGACGAGTACCGCTATCGCTGGTGAATTAAGGCCGTGTCAGTGGAGGAG

MCU in exon 10 of PTM

88 OF 192 (46%) bases in PTM exon 10 are not complementary to
its binding domain (bold and underlined).

FIG.31

Sequence of a double
Trans-spliced product

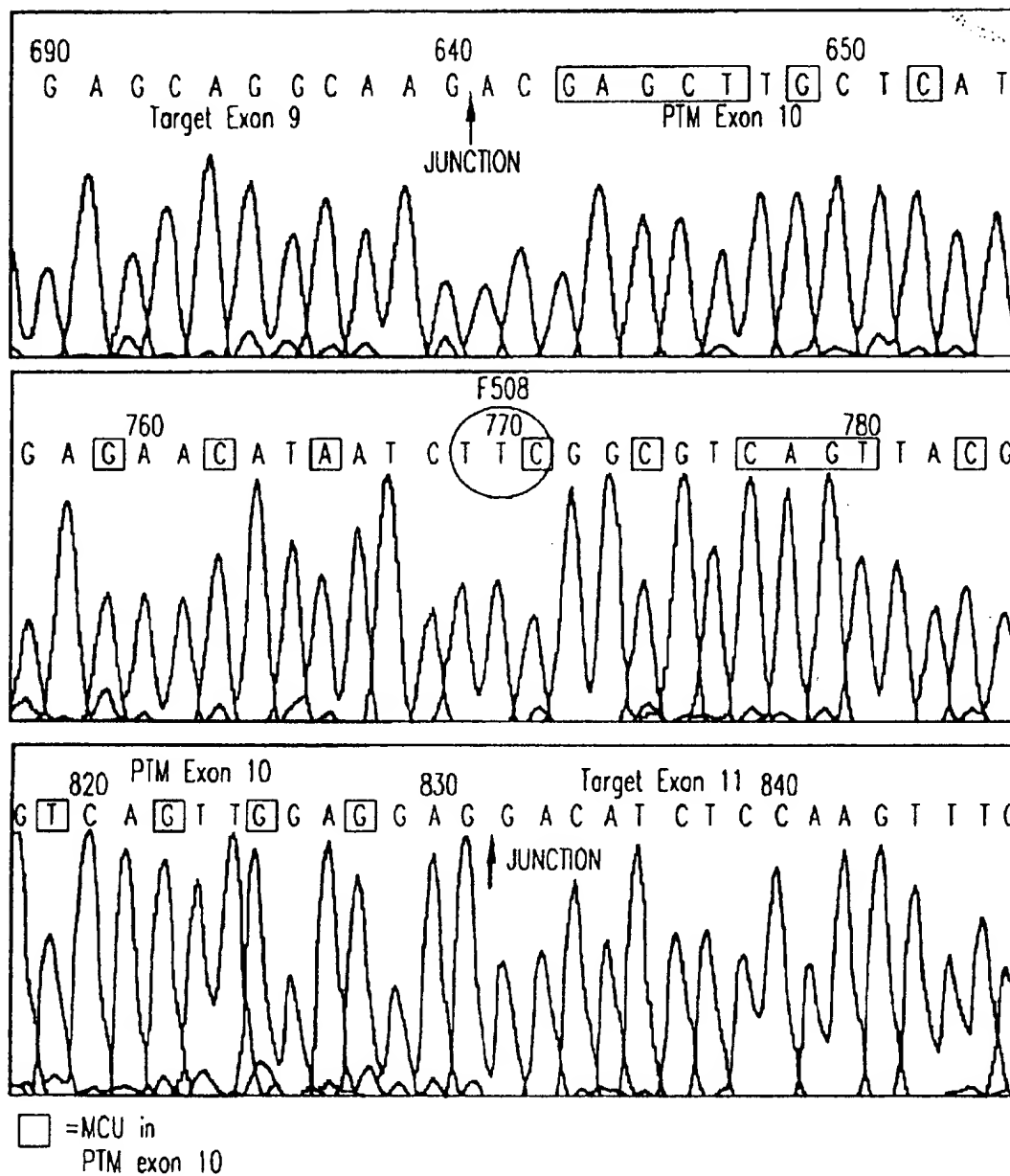


FIG.32

CF-TR Repair: 5' Exon-Replacement schematic diagram of a PTM binding to the splice site of intron 10 of a mini-gene target

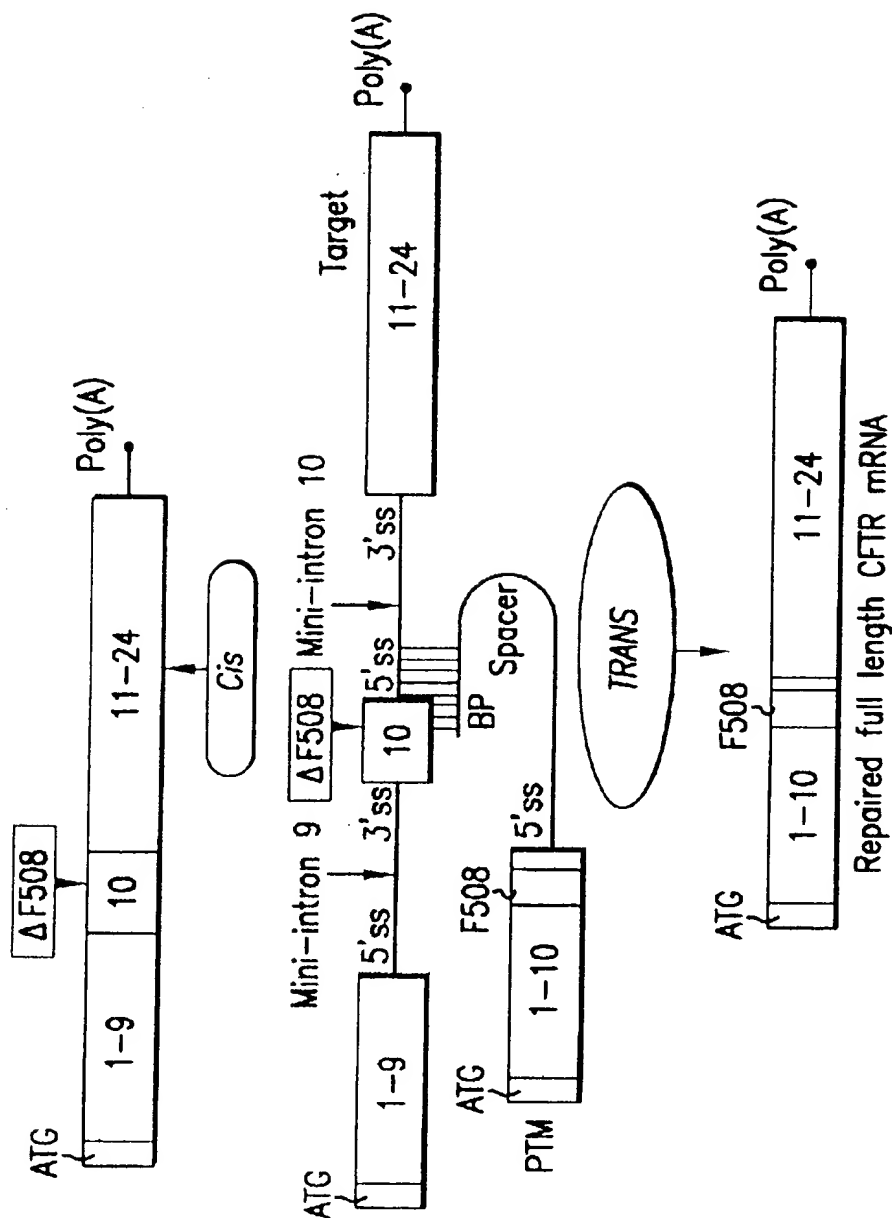


FIG.33

PTM with a short binding domain masking a single splice site in a mini-gene target.

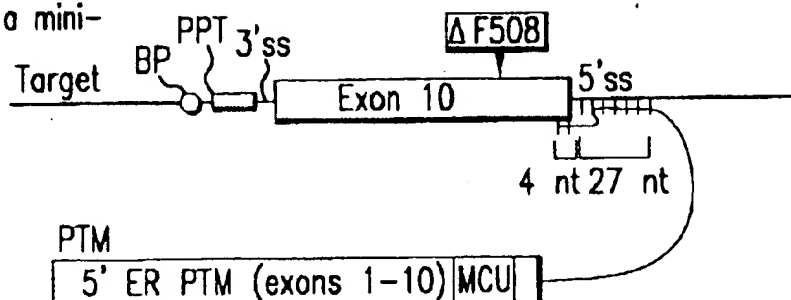


FIG.34A

PTM with a long binding domain masking two splice sites in a mini-gene target.

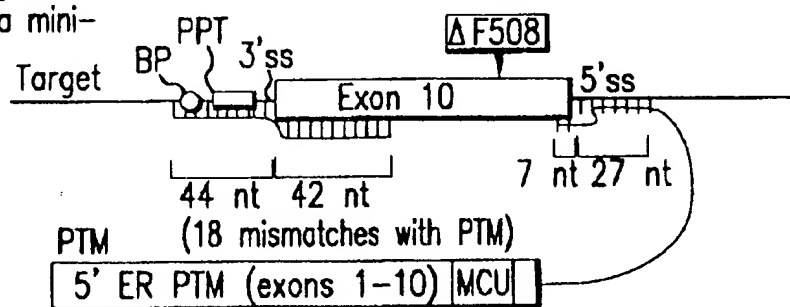


FIG.34B

PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini-gene target.

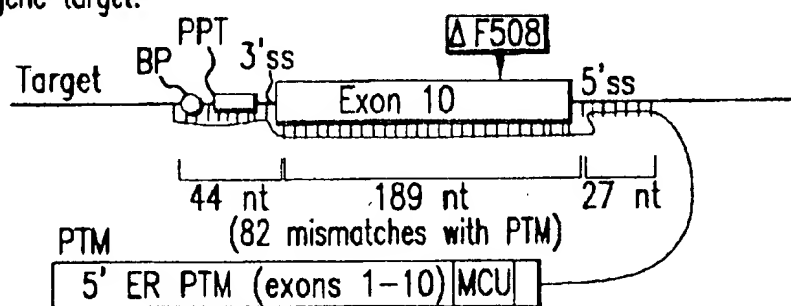
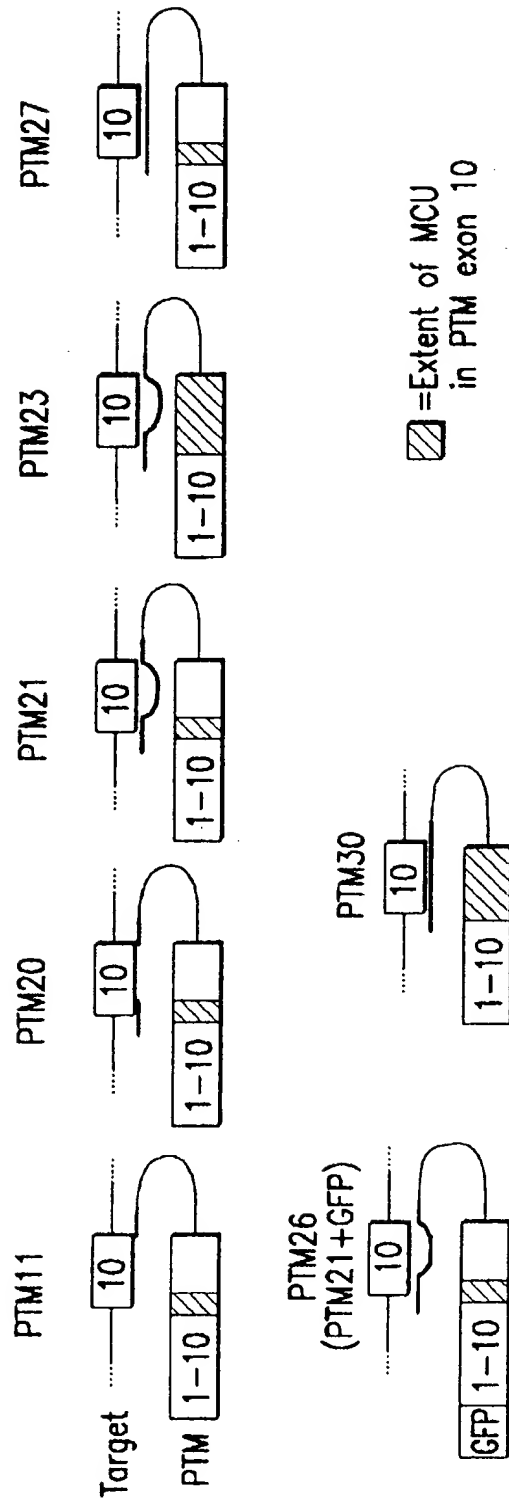


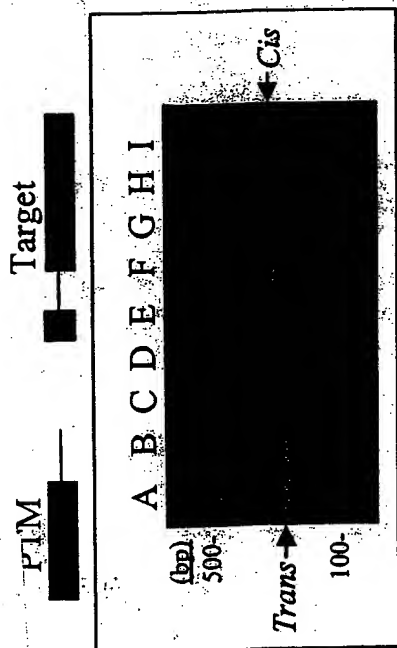
FIG.34C



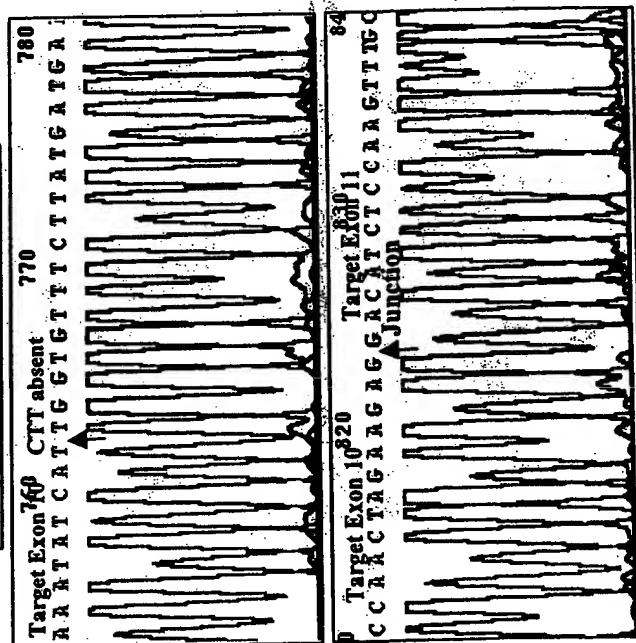
MCU in exon 10 of PTM
88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain.

ACGAGCTTGCITCATGATCATGGCGGAGTTAGAACCACAGTCAAGCCAAAGATCAAACATTCGG
GCCCATCAGCTTTTCAGCCAAATTCAGTTGGATCATGCCCGGTACCATCAAGGAGAACTAAT
CTTGGCGGTCAGTTACGACCGAGTACCGCTATCCCTGGTGAATAAGGCCCTGTCAGTTGGAGGAC

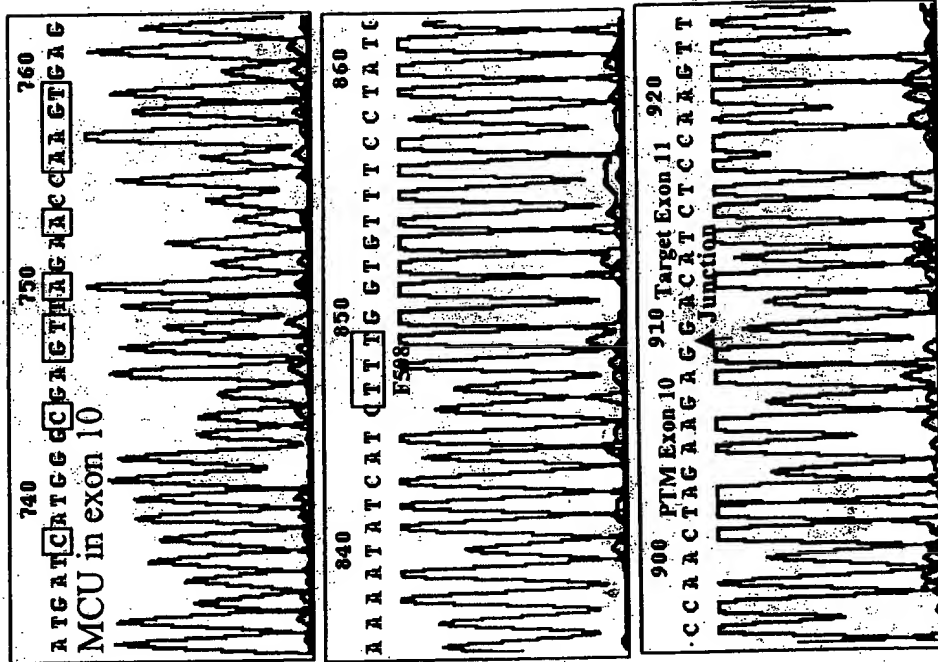
FIG. 35

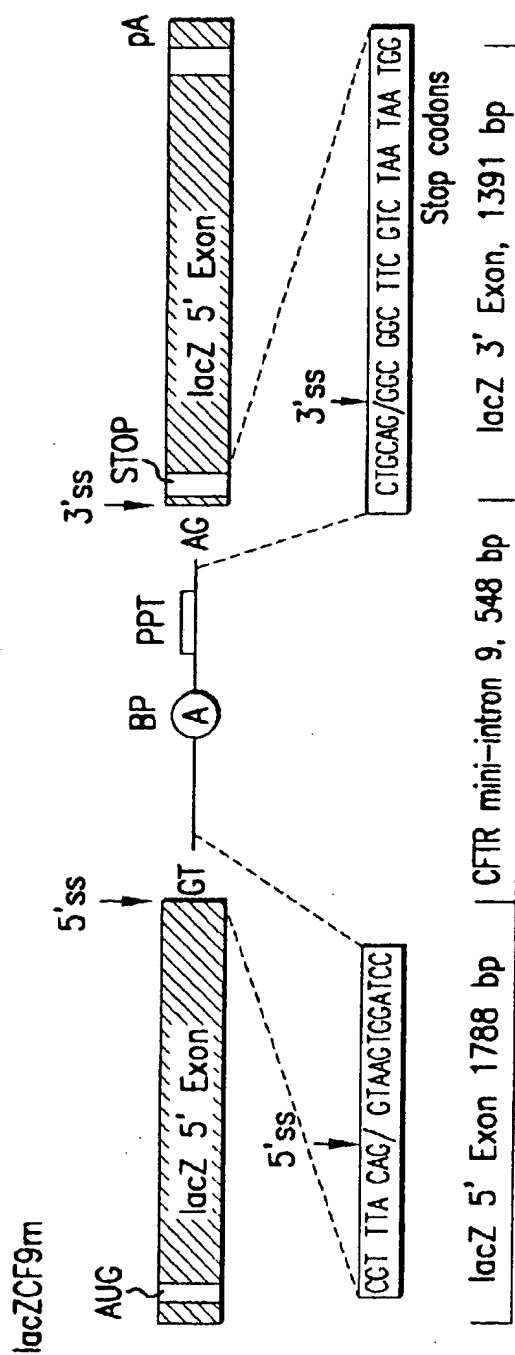


A. *Cis-spliced product* [Primers CF1 + CF111]



B. *Trans-spliced product* [Primers CF93 + CF111]





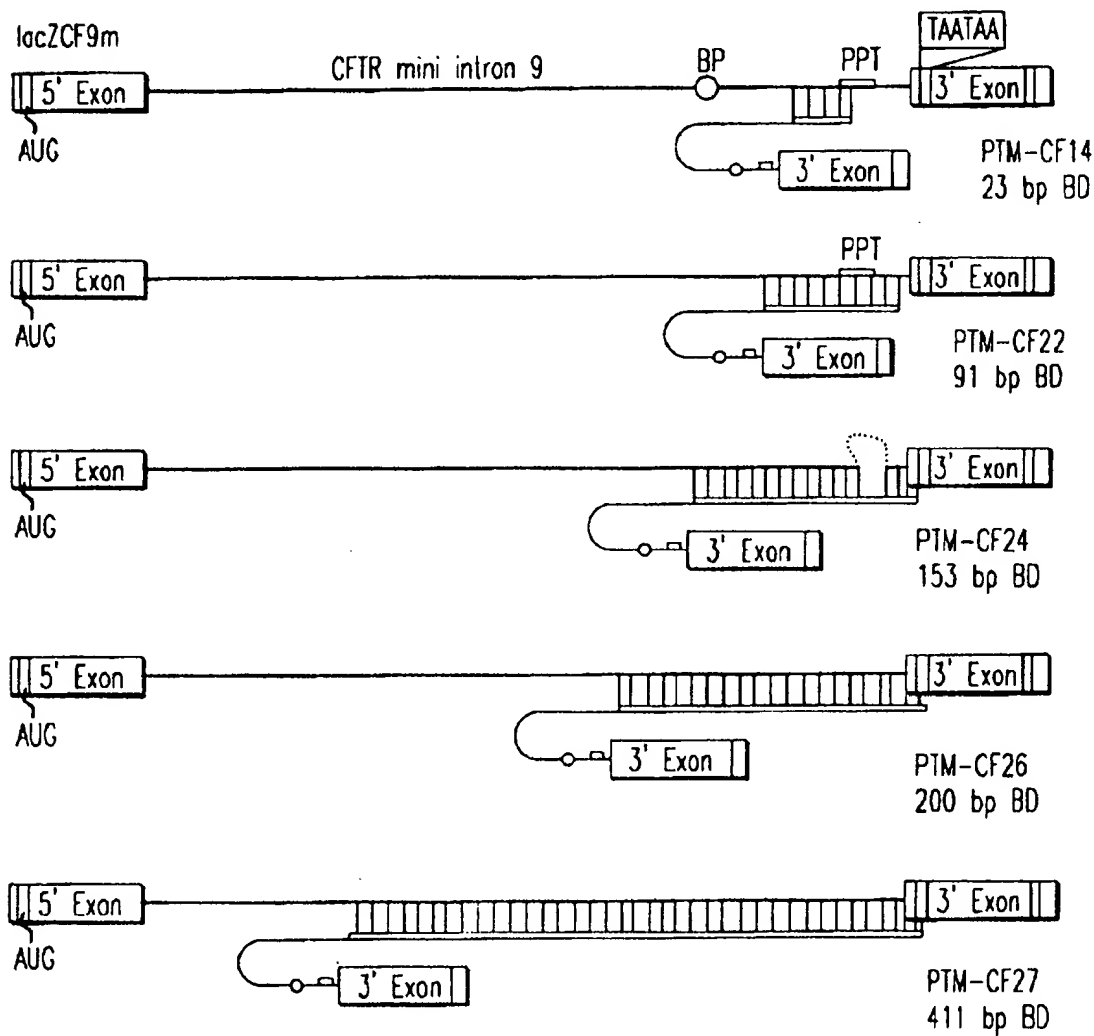
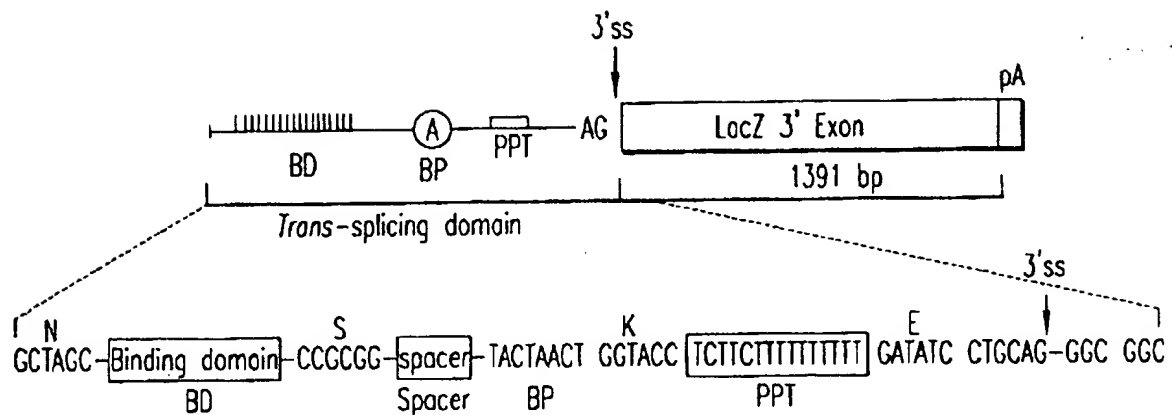


FIG.37B

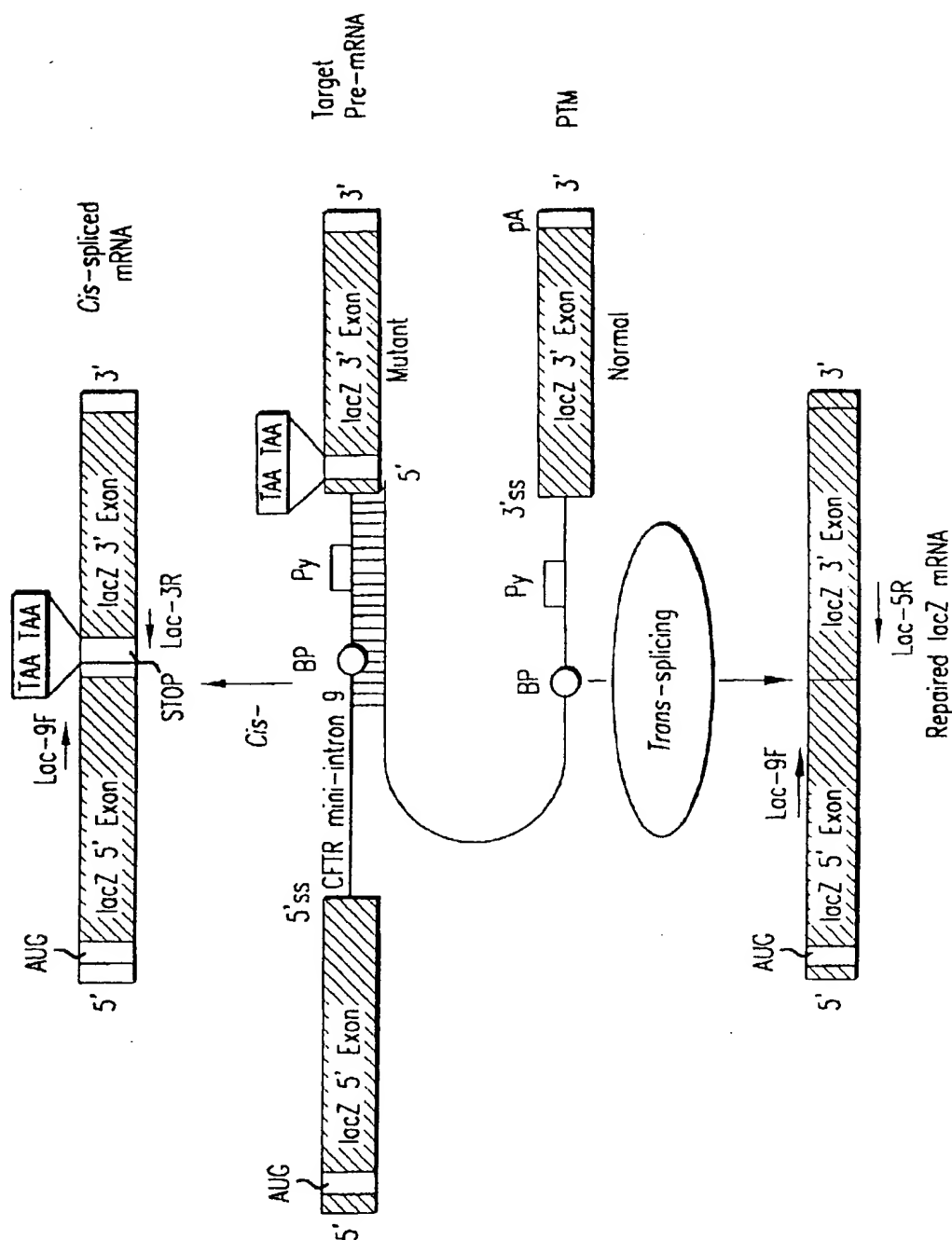


FIG.37C

200703060000

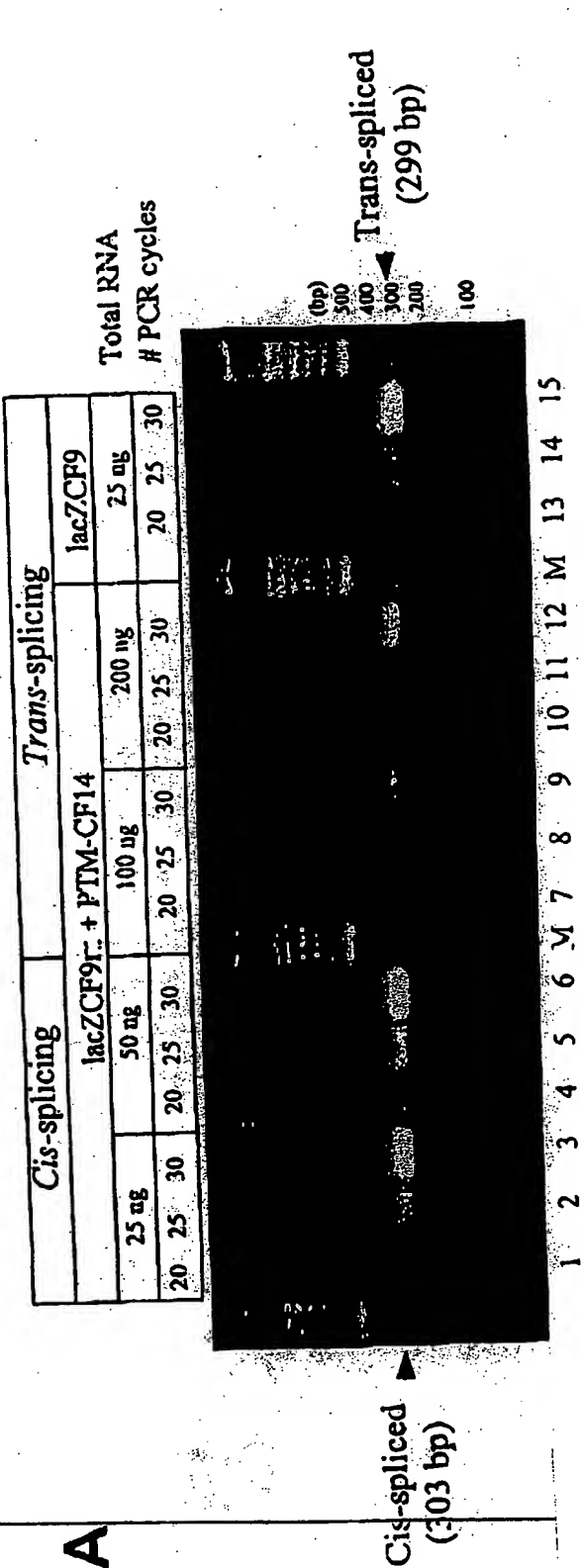
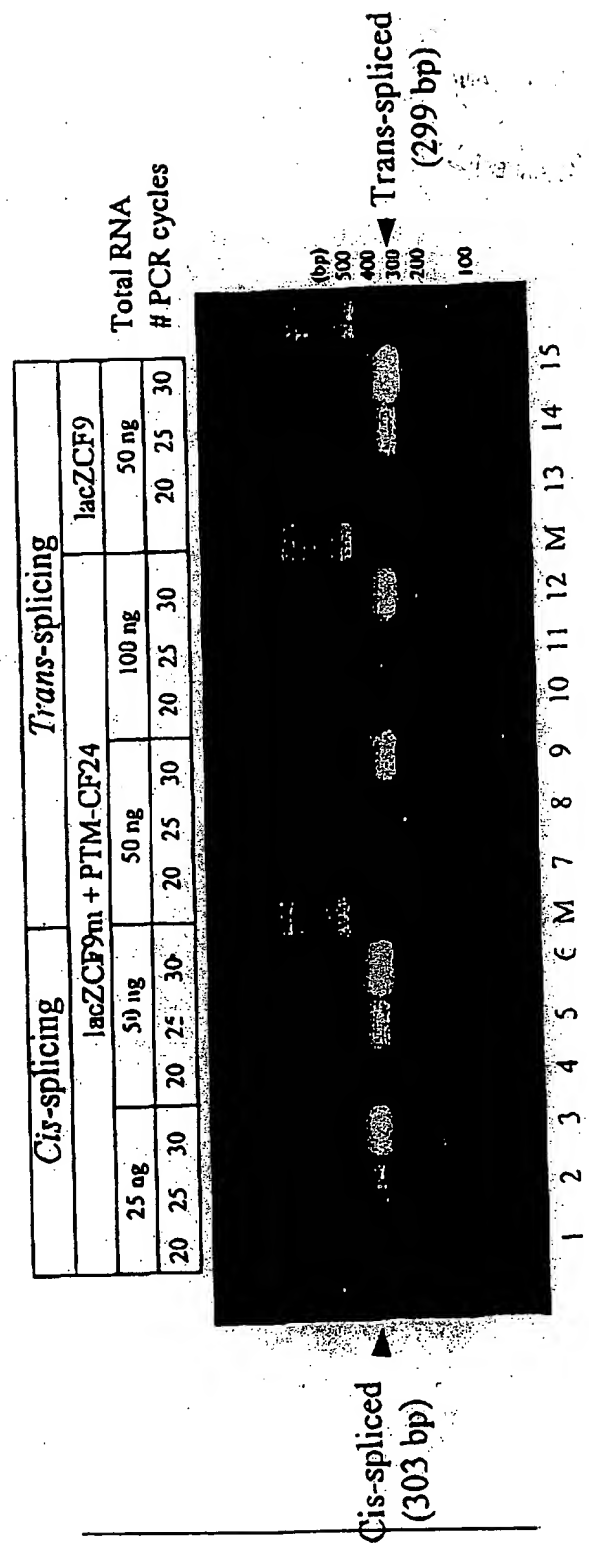


Figure 38A



B

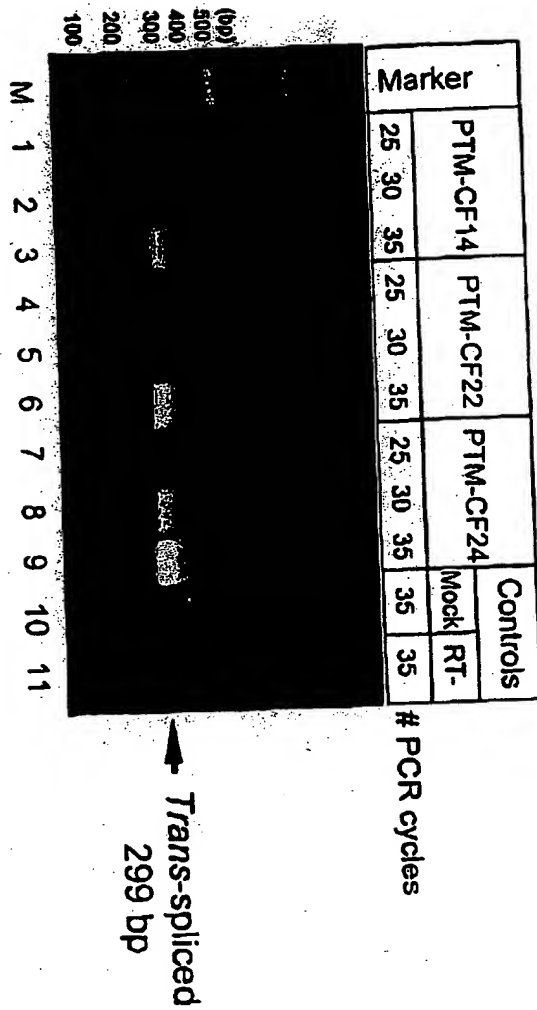


Figure 38B

(b)



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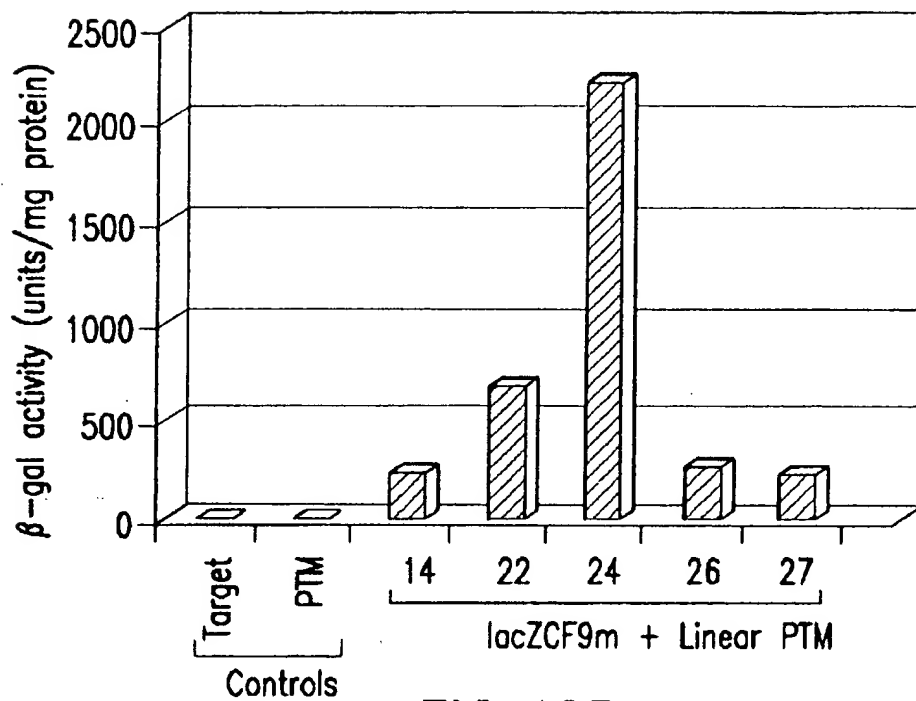


FIG.40B

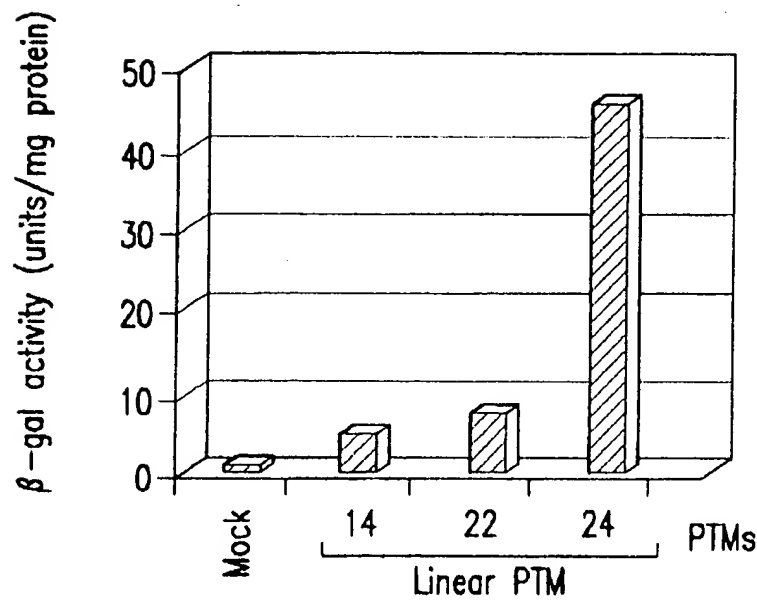


FIG.40C

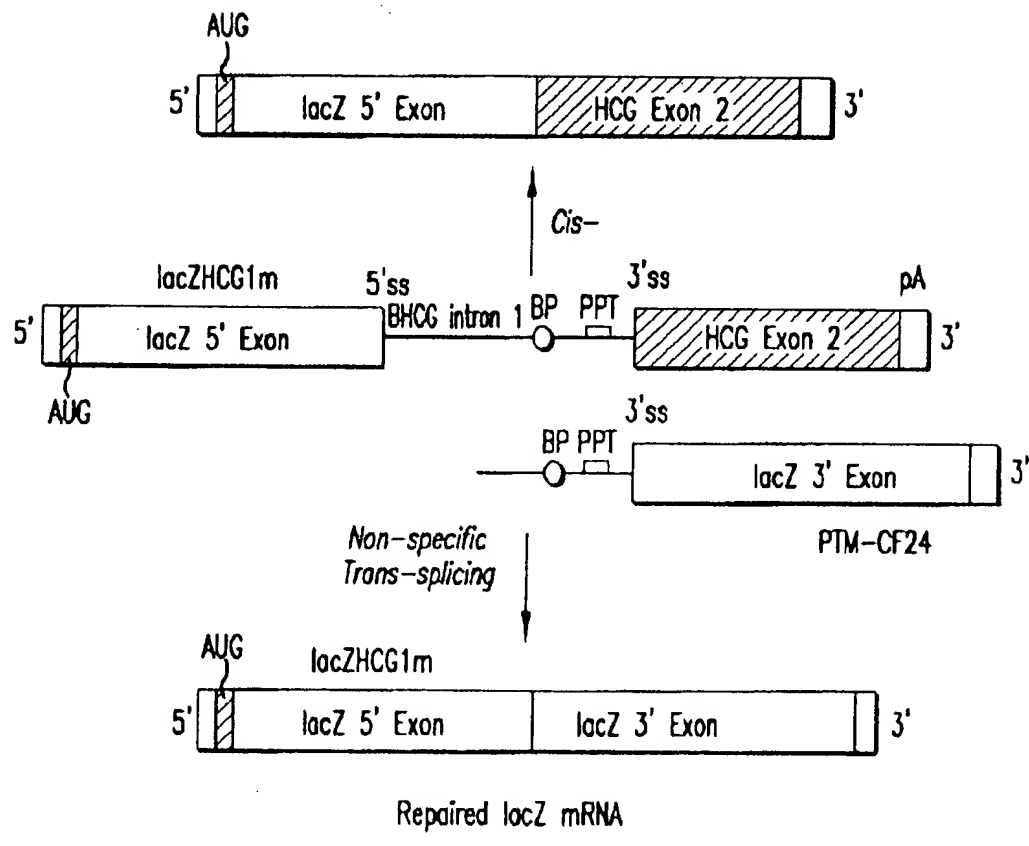


FIG.41A

1000

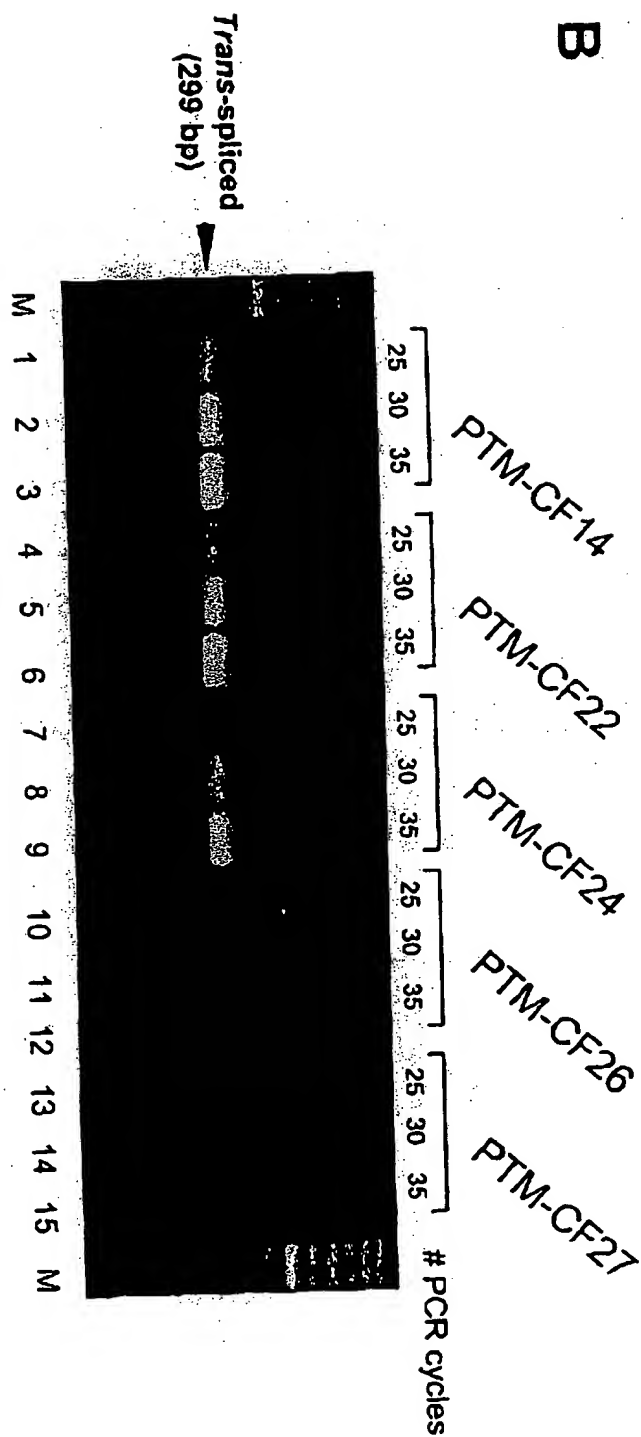
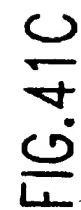


Figure 4KB



[illegible]

153 bp BD underlined

TTC TTATT TGTAA GATTCTA TTA ACTCAT TTGAT TCAAA ATATTT AAATACT TCCTG TTTCACCT ACTCTGCTATGC

AC-CCGCG

FIG. 43A

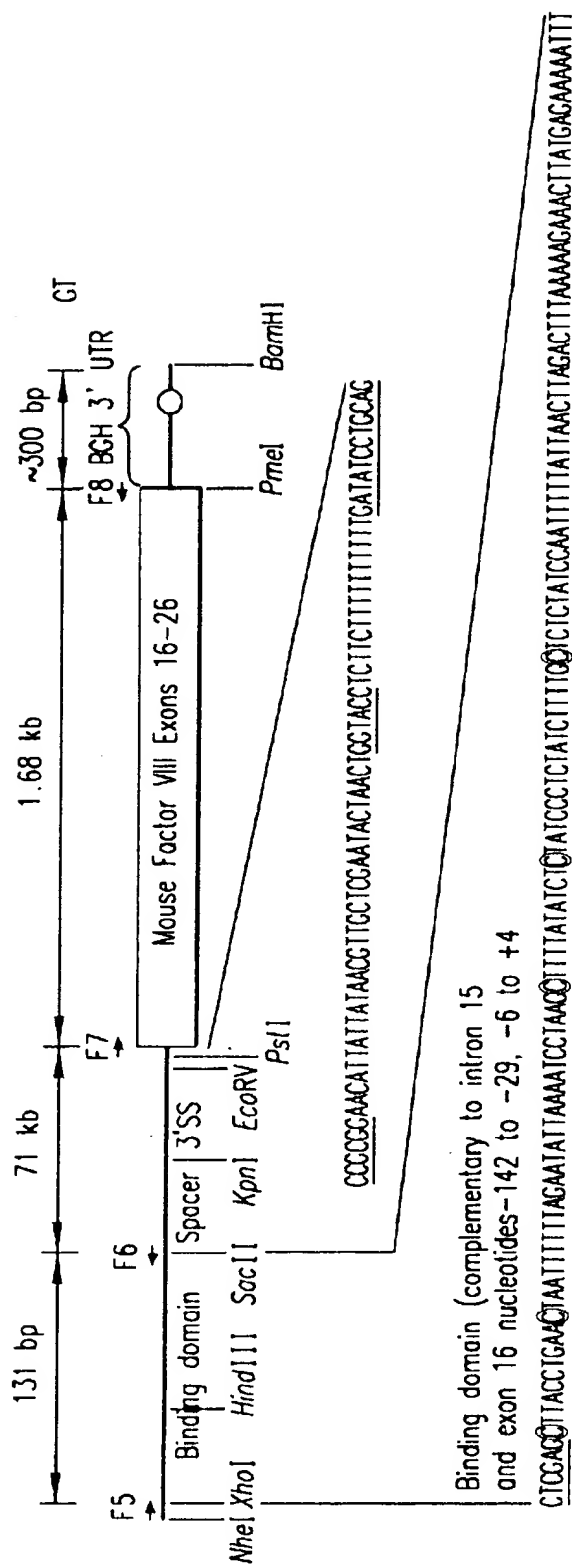
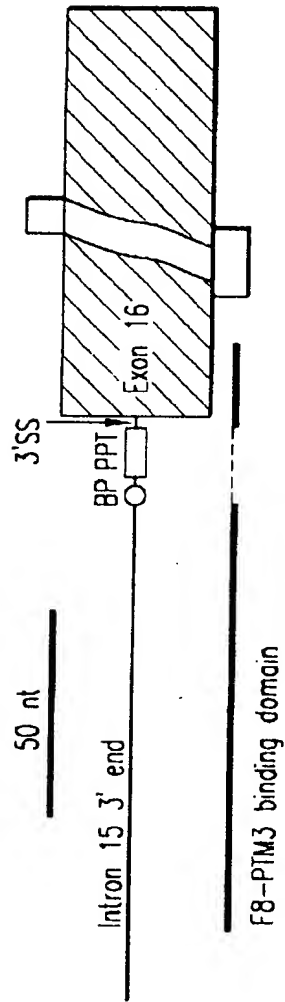


FIG. 44A

FIG. 44B



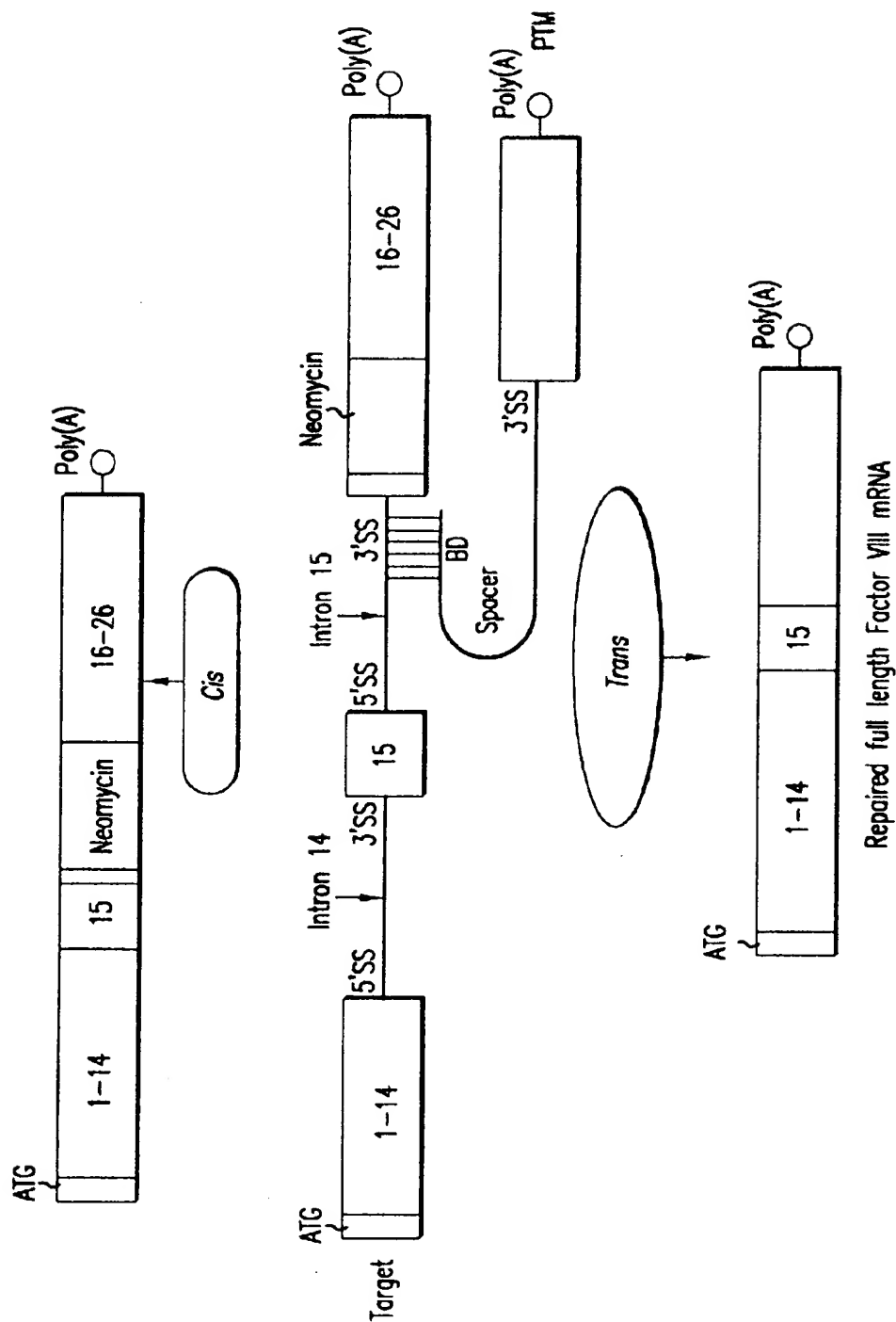
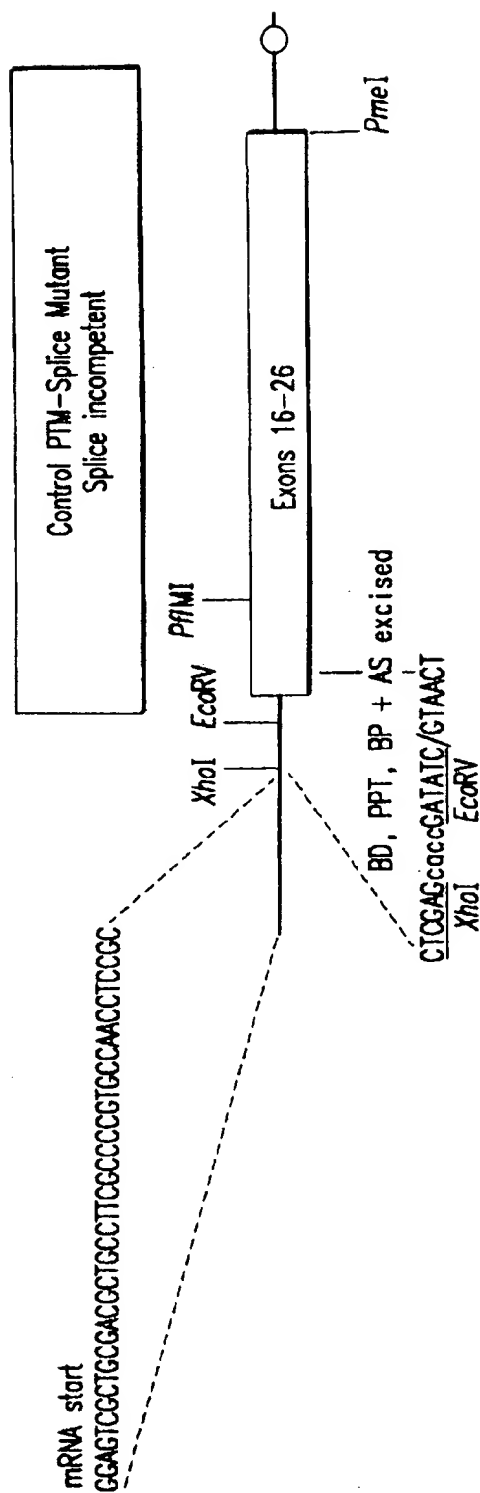


FIG.44D



Method:

Excise TSD and part of exon 16 with XhoI and PfuMI and ligate in a PCR product that:

- 1) eliminates the TSD and splice acceptor site
- 2) inserts EcoRV adjacent to exon 16
- 3) restores the coding for exon 16

FIG.45

Repair of Factor VIII
Preliminary results from one experiment

FVIII activity in Exon 16 FVII-KO mice
after IV PTM-FVII intraportal infusion
(100 μ gDNA)(n=3)

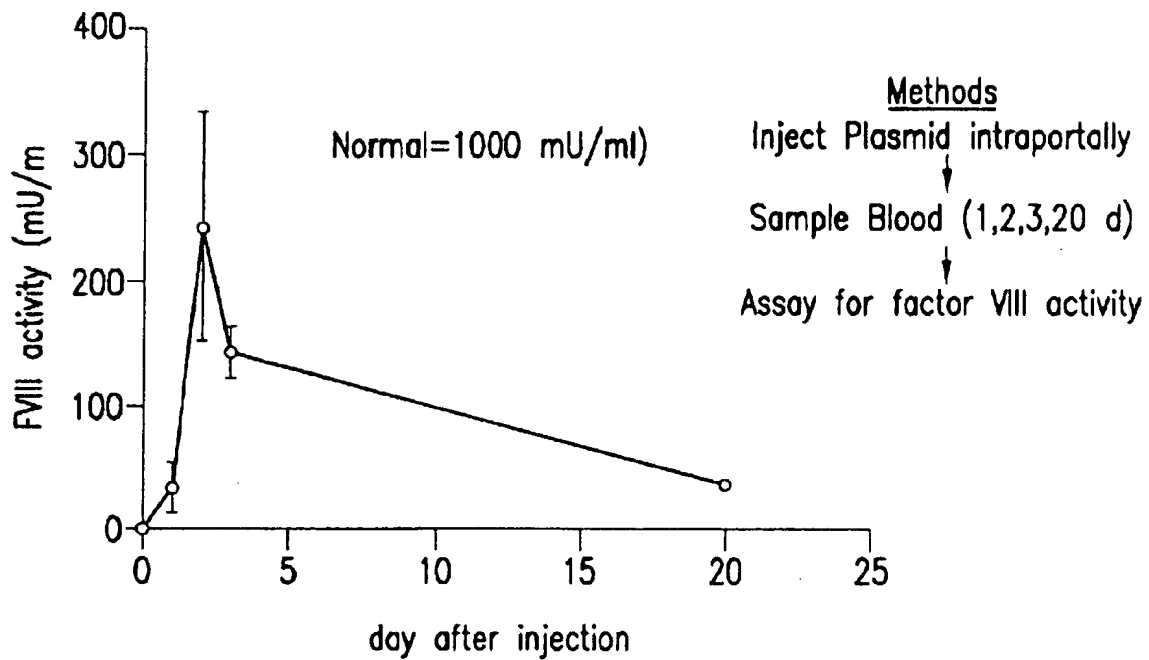


FIG.46

Detailed structure of a mouse factor VIII PTM containing normal sequences for exons 16-26 and a C-terminal FLAG tag. BGH=bovine growth hormone 3' UTR; Binding domain= 125 bp.

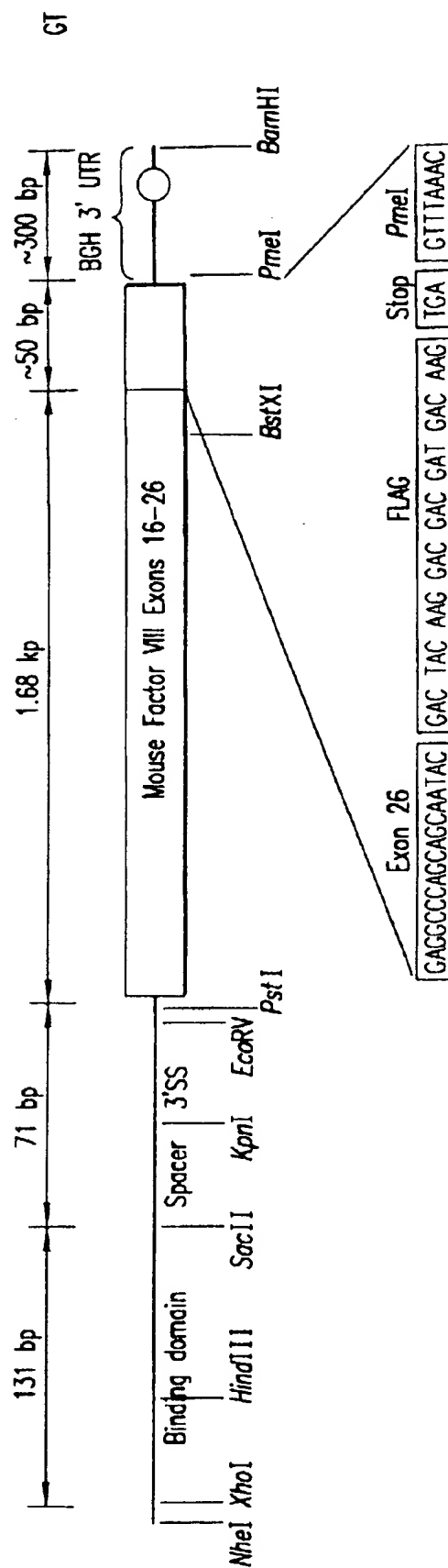
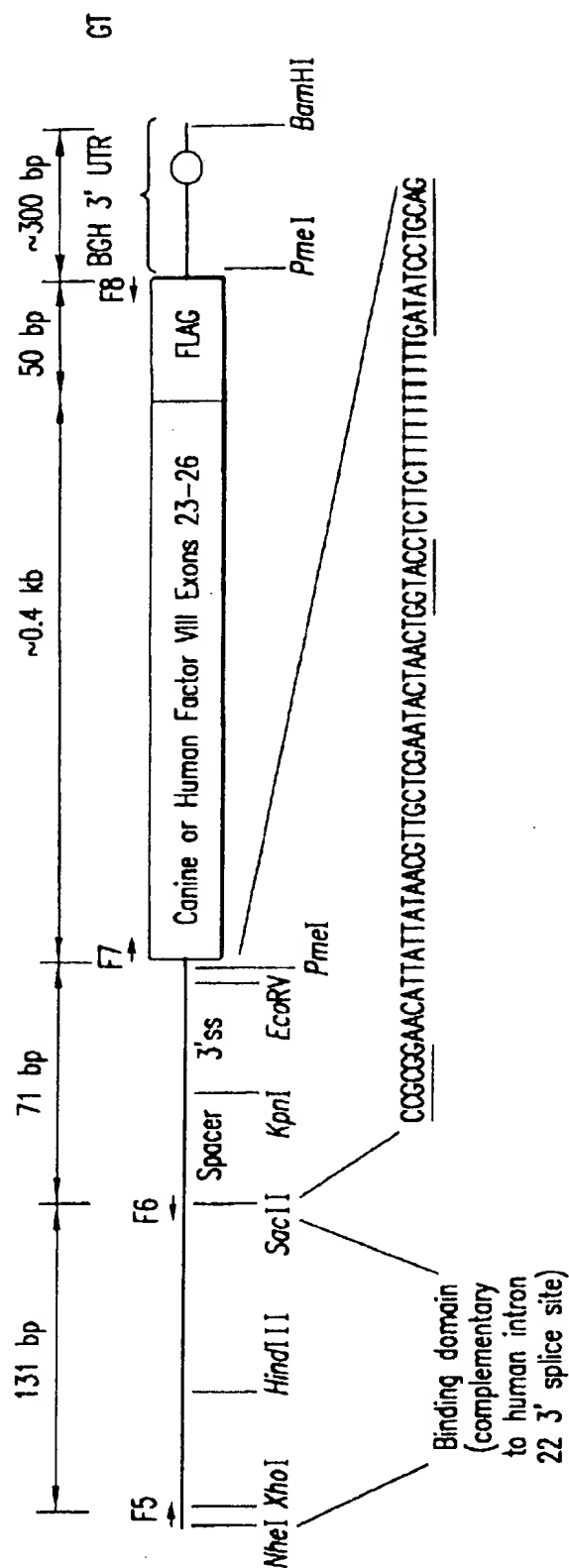


FIG.47A



FLAG=C-terminal tag to be used to detect repaired factor VIII protein.

FIG.47B